The Journal of Laboratory and Clinical Medicine

VOL. XV ST. LOUIS, MO., JANUARY, 1930 NO. 4

AMERICAN SOCIETY of CLINICAL PATHOLOGISTS

RECENT DEVELOPMENTS IN TULAREMIA (FRANCIS' DISEASE)*

WITH A REPORT OF ELEVEN ADDITIONAL CASES

BY WALTER M. SIMPSON, M.S., M.D., F.A.C.P., DAYTON, OHIO

TULAREMIA has abruptly become a common and widely-recognized disease of man. Up to 1924, but 15 cases of tularemia in human beings had been reported. During the past five years, over 800 proved cases have been recognized in the United States. Cases have now been reported from every state in the Union, except the New England States, Delaware and Washington. The incidence ends abruptly at the Canadian and Mexican borders, despite the large number of cases discovered in border states.

Ohio ranks first among the states, with 92 recorded cases. Montana ranks second with 66 cases. Forty-five cases have been found in Washington, D. C. It is not without significance that the largest number of cases have been found in those states in which certain individuals have been actively engaged in investigating the disease. The obvious inference is that many more cases would be unearthed in other localities if special effort were made to find them.

For many years it appeared that tularemia was restricted to the United States. Recent reports indicate that it is probably a world-wide disease. Francis and Moore[1] demonstrated that "Ohara's disease" in Japan is identical with tularemia. Three laboratorians in London, England, developed the disease after performing autopsies on laboratory animals inoculated with cultures of *Bacterium tularense* sent by Francis at their request.[2]

Four recent contributions by Russian workers, published during 1928, indicate that tularemia is probably widespread in the Union of Socialistic

*From the Diagnostic Laboratories of the Miami Valley Hospital, Dayton, Ohio.

Read, in part, before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.

This paper received the Ward Burdick Research Award for the year 1929.

Soviet Republics. Nikanorov[3] tells of three extensive outbreaks of a "tularemia-like" disease. The first occurred in the province of Astrakhan, involving about 150 persons. The second large group of cases (over 100) was encountered in the province of Uraisk. Simultaneously, a tremendous number of cases (over 800) was discovered in the province of Riazan, on the Oka River.

All of the Russian cases resulted from direct contact with the fur-bearing water rat or water vole of Europe *(Arvicola amphibius)* which is hunted for its fur. The onset of the epidemics in nine villages along the Oka River was associated with a flood which drove the water rats ashore in great numbers. The furs of these animals have considerable commercial value and the inhabitants of that region seized upon the opportunity to obtain large numbers of the animals with relatively little effort. The water rats are ordinarily pierced with a harpoon, which enters the body of the rat very readily but is withdrawn with great difficulty. The hands of the hunters are always covered with blood during the process of releasing the harpoon and skinning the animals.

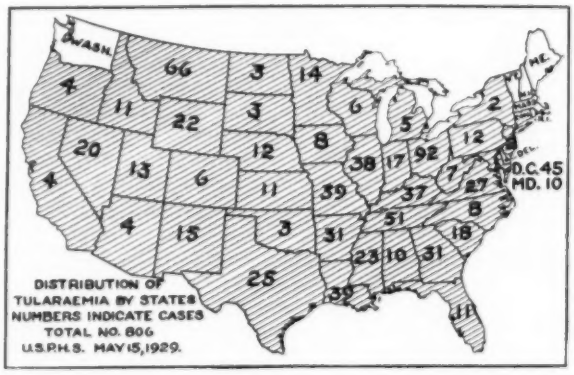


Fig. 1.

A later report by Suvorov, Wolferz and Voronkova,[4] from the antiplague laboratory of Astrakhan tells of 200 cases among human beings in that province. The epidemic reached its height in June, 1928. There were no deaths. Guinea pigs, white mice, and ground squirrels died, with typical autopsy findings of tularemia, after inoculation with material from the suppurating lymph nodes of human beings. A gram-negative, nonmotile coccus was recovered on cystine-glucose agar. These organisms were agglutinated by the serum of convalescent patients in dilutions of 1:800.

In the spring of 1928, Golov, Kniazevsky, Berdnikov and Tiflov[5] encountered 105 cases in eight villages situated along the Oural River. These cases also developed as the result of the handling of water rats. Guinea pigs inoculated with the contents of the enlarged regional lymph nodes died in eight days; autopsy revealed multiple yellowish-white foci of necrosis in the spleen and liver. These workers captured and examined 96 water rats; three were found dead. Thirty-eight of the animals showed either greatly enlarged spleens (18), enlarged caseous lymph nodes (6), multiple encapsulated subcutaneous abscesses (9), suppurating inguinal buboes (2), foci of necrosis in the liver and spleen (2), or necrotic nodules in the lungs (1). Smears from

the necrotic lesions revealed a gram-negative coccobacillus. Three guinea pigs died after inoculation with material from some of the lesions, showing the characteristic pathologic anatomy of tularemia. From these lesions an organism, regarded by the writers as identical with *Bacterium tularense,* was recovered on coagulated egg-yolk media. Guinea pigs and white mice injected with the second generation of organisms died in three to five days following inoculation, with the production of the same gross lesions of the spleen, liver and lymph nodes, and yielding the same growth on egg-yolk media. The organisms thus recovered agglutinated in high titer in the serums of human convalescents.

These ardent investigators paid the inevitable toll for their discoveries. Four Russian physicians acquired the disease during the course of these investigations, thus bringing the total number of laboratory infections with tularemia to twenty-four.

Professor Zarhi, of Sverdlovsk (Ekaterinburg) in the province of Perm, Russia, sent serum of a patient thought to have acquired tularemia, to McCoy, at the Hygienic Laboratory at Washington. McCoy found that it agglutinated *Bacterium tularense;* he also isolated *Bacterium tularense* from guinea pigs which had been inoculated with guinea pig spleen tissue received at the same time from Professor Zarhi.

New animal and insect hosts and transmitters of tularemia have been recently discovered. Dieter and Rhodes[6] found that tularemia exists in nature among wild rats which were trapped in Los Angeles, California. Perry[7] isolated *Bacterium tularense* from meadow mice *(Microtus californicus aestuarinus)* in Contra Costa County, California.

Parker and Dade[8] found evidence of present or recent infection with *Bacterium tularense* in eight sheep *(Ovis aries)* of a large Montana band, a considerable percentage of which were affected by a similar illness; Parker is of the opinion that many of the heavy losses among sheep in that region have been due to tularemia. The sheep were known to be infested with the wood tick *(Dermacentor andersoni Stiles)*—a frequent host and transmitter of tularemia. Parker and Francis succeeded in recovering *Bacterium tularense* from the spleens of guinea pigs which had been inoculated with wood ticks found on the sick sheep; the organism was likewise recovered from sheep tissues.

Green, Wade and Dewey[9] had demonstrated that the muskrat *(Ondatra zibethica)* is experimentally susceptible to tularemia, but it is only within the past few months that Schwartz,[10] of Montana, has reported the development of tularemia in two Japanese section laborers who had cooperated in skinning a muskrat. Mease,[11] of Florida, has reported a case in a human being of tularemia resulting from the skinning of opossums *(Didelphis virginiana).*

A case in a human being of oculoglandular tularemia resulting from contact with a woodchuck *(Marmota flaviventer;* ground hog) is included in the series of cases reported in this communication. The only other reported case of tularemia in a human being as the result of direct contact with a naturally infected woodchuck was reported to Francis[12] by Dr. J. T. Powell, of Gravette, Arkansas. A fifty-eight year old man cut his finger while skinning a wood-

chuck which he had killed. Death occurred twenty-three days after the injury. The patient's blood serum agglutinated *Bacterium tularense* in all dilutions to 1:1280.

Even though McCoy,[13] McCoy and Chapin,[14] and Wherry[15] found the domestic cat *(Felis catus)* to be unaffected by inoculations with *Bacterium tularense*, Green, Wade and Hanson[16, 17] have accumulated evidence which would seem to incriminate the cat as an occasional naturally infected carrier. Francis,[18] attempted to infect thirteen cats with tularemia; two kittens died of the disease; the other animals did not acquire the disease. Francis places the cat among the mildly susceptible animals.

I[19] found dogs to be naturally immune to the disease.

The repeated observations of laymen and scientific investigators of the simultaneous decimation of wild rabbits and game birds in certain localities led to the suspicion that tularemia may have been the common cause. This possibility was strengthened by the discovery, by Parker and Spencer,[20] that the common rabbit tick *(Hemaphysalis leporis-palustris)*, an important transmitter of tularemia from rabbit to rabbit, is also found on game birds. Parker and Spencer have produced tularemia experimentally in the blue grouse of Montana. Green and Wade[21] have found the ruffed grouse of Minnesota to be just as susceptible to experimental inoculation with *Bacterium tularense* as are guinea pigs and rabbits.

Parker[22] recovered *Bacterium tularense* from guinea pigs which had been inoculated with the tissues of five quail *(Colinus virginianus;* bobwhite) which had died after the ingestion of food contaminated with tularemia-infected tissue. Parker concluded that quail may at least be considered potential agents of human infection with tularemia. A recent report of natural infection of quail with tularemia by Green and Wade[23] lends support to Parker's beliefs.

The deer fly *(Chrysops discalis)* and the wood tick *(Dermacentor andersoni* Stiles) are common transmitters of tularemia in the northwestern states. Another tick, thought to be *Dermacentor variabilis*, has been the responsible transmitting agent in 25 human cases of tularemia in Arkansas, Oklahoma, Texas, Louisiana, Tennessee and Minnesota. Parker, Brooks and Marsh[24] have recently recovered *Bacterium tularense* from wood ticks of the species *Dermacentor occidentalis* Newman, collected in San Benito County, California. Even though new animal hosts and insect vectors are being constantly discovered, emphasizing the ever-widening dissemination of the disease, the wild rabbit is by all odds the most important reservoir of infection.

DAYTON EXPERIENCE WITH TULAREMIA

In June, 1928, I[25] reported 48 proved nonfatal cases of tularemia in the human being, and one rapidly fatal case, occurring in Dayton, Ohio. A detailed report of the clinical, pathologic, bacteriologic, and serologic findings in the most rapidly fatal case of tularemia on record (four days, seven hours), together with a description of the experimental production of the ulceroglandular, oculoglandular and glandular forms of the disease in laboratory animals, appeared somewhat later.[26]

Bacterium tularense was grown directly from human tissues on artificial culture media for the first time; a human serum modification of Francis' cystine-glucose-meat infusion-peptone agar was employed.

It was demonstrated that tularemia had been prevalent in Dayton for twenty years, and that during that time seven deaths, in all probability due to tularemia, had occurred.

In November, 1928, four additional Dayton cases were reported.[27]

One purpose of the present undertaking is to report 11 recently discovered cases, thus bringing the total of the cases investigated by me to 64, sixty of which originated in or near Dayton, Ohio. This is by far the largest number of cases to be recorded from such a circumscribed area.

The pertinent clinical and serologic findings in the newly discovered cases follow:

CASE 1.—Mr. G. S., a forty-year-old farmer, skinned and eviscerated a young ground hog (woodchuck) which his dog had caught while on an expedition through the woods with his boys, on May 15, 1928. During the process of eviscerating the ground hog, Mr. S.'s son, while playing with the liver of the ground hog ruptured the gall bladder and some of the bile entered Mr. S.'s right eye. Four days later the eye became intensely swollen and infected. On June 12, 1928, Dr. J. W. Payne, health commissioner of Lawrence County, Ohio, examined the patient and "found the eye very much swollen, conjunctiva red as fire, multiple small yellowish areas on both palpebral conjunctivae, considerable cellulitis with edema about the right eye, and painful enlargements of the parotid and submaxillary lymph nodes. The submaxillary lymph nodes finally suppurated and broke through the skin about August 1. The patient experienced repeated chills, night sweats, and hot flashes. The bones of the head and upper extremities were extremely painful. The patient was unable to resume his duties until October, 1928."

Serum of Mr. S., submitted to George W. McCoy of the Hygienic Laboratory at Washington, D. C., on January 14, 1929, was found to agglutinate *Bacterium tularense* in all dilutions to 1:160. Similar results were obtained by me.

CASE 2.—Mrs. L. B., patient of Dr. E. E. Bohlender, a thirty-six-year-old housewife, cleaned three wild cottontail rabbits which had been killed by her husband in Marion County, Indiana, on November 1, 1928. While dressing the rabbits she cut the left index finger with a knife. Two days later she experienced a sharp chill followed by a rapid elevation of temperature (104° F.) She went to a physician in Indianapolis who made a diagnosis of influenza. On the second day of her illness she observed that the left axillary lymph nodes were enlarged and painful. During the next two weeks she experienced repeated chills, and sweats, accompanied by feverishness, headache, backache, muscular and joint pain. During the first week of her illness she was moved to Dayton, where Dr. Bohlender recognized the case at once as one of tularemia.

Serum of Mrs. B., collected on November 15, 1928, was submitted to the Hygienic Laboratory at Washington, D. C., where it was found to agglutinate Bacterium tularense in all dilutions to 1:320, with partial agglutination in a titer of 1:640.

Mrs. B. remained bedfast for two weeks after which the fever gradually disappeared and she made an uneventful recovery. The axillary lymph nodes did not suppurate but slowly regressed in size over a period of five months.

CASE 3.—Miss A. G., patient of Dr. H. H. Stafford, aged thirty-two, dressed three wild cottontail rabbits which had been killed by friends in Preble County, Ohio, on November 15, 1928. Miss G. had scratched the skin over the proximal phalanx of the right thumb two days before this. On November 17, she developed repeated chills with a fever of 104° F. She thought that she "had a bad case of grippe." She remained in bed for one week, during which time she experienced repeated chills and sweats. On the third day of her illness she noticed for the first time a small ulcer which had developed at the site of

the scratch on the right thumb. The same day she noticed a painful swelling of the right axillary lymph nodes. The axillary mass did not suppurate.

When examined by me on December 1, 1928, Miss G. was afebrile, a tiny healing ulcer was observed on the right thumb, and the right axillary lymph nodes were walnut-sized and of firm consistency; suppuration did not occur. Convalescence was uneventful.

Serum of Miss G., collected on December 1, 1928, was found by George W. McCoy, of the Hygienic Laboratory, and me, to agglutinate *Bacterium tularense* in all dilutions to 1:160, with partial agglutination at 1:320.

CASE 4.—Mrs. A. J., aged sixty-six, patient of Dr. D. C. Casto of Parkersburg, West Virginia. Her son, a resident of Dayton, came to my office on November 25, 1928, with the information that he had just received a letter describing his mother's illness and that he was certain from the description that his mother was a victim of "rabbit fever." He recited the details of his mother's illness and they justified his suspicions. I gave him a tube and a mailing container and insisted that he mail me a blood specimen as soon as he reached Parkersburg.

Serum of Mrs. J., collected on December 1, 1928, was found by George W. McCoy, of the Hygienic Laboratory, and me, to agglutinate *Bacterium tularense* in all dilutions to 1:1280. In the routine test for agglutination of *Bacterium abortus* it gave a positive reaction in all dilutions to 1:80.

Through the courtesy of Dr. Casto and as a result of an interview with the patient, who later visited Dayton, the following history was obtained: Mrs. J. dressed some forty or fifty wild cottontail rabbits which had been shot by her husband on their farm, during the week preceding the development of her illness. On November 16, 1928, while dressing rabbits, she perforated the skin of the right index finger with a needle-like fragment of rabbit bone. On November 20, she experienced repeated chills, high fever, profuse sweats, aching pains in the head and extremities, accompanied by marked prostration. The original diagnosis was influenza. The right axillary lymph nodes rapidly enlarged to orange-size.

A small circumscribed ulcer developed at the site of the perforation of the skin of the right index finger. This was incised without improvement on November 30.

Mrs. J. remained bedfast for four weeks, during which time she experienced repeated chills and sweats and lost considerable weight.

On January 2, 1929, Dr. Casto incised the suppurating mass in the right axilla and two or three ounces of thick exudate escaped. A sinus tract has persisted at the site of the incision up to the present time (June 10, 1929).

Mrs. J. was unable to perform her household duties for three months. When examined by me on May 2, 1929, she appeared to be in good health. She has regained all of the weight which was lost during the acute illness.

Serum of Mrs. J., collected by me on May 6, 1929, was found to agglutinate *Bacterium tularense* in the same dilution as before (1:1280), with cross agglutination of *Bacterium abortus* in a dilution of 1:20. It is interesting to observe that the tularemia titer remained the same over a period of four months.

CASE 5.—Mr. F. C. D., patient of Dr. J. T. Mackie, a twenty-five-year-old gasoline station operator, killed five wild cottontail rabbits in the woods near Dayton on November 15, 1928. While climbing a fence during the hunt, he cut the skin of the palmar surface of the proximal phalanx of the left middle finger on a wooden post. While dressing the rabbits at home that evening, he contaminated this fresh cut with rabbits' blood. On the evening of November 17, he experienced a feeling of soreness in the region of the left elbow. On November 18, he observed a walnut-sized mass in the epitrochlear area; during his work on that day he suffered from chilliness, feverishness, and weakness. The next day the fever reached 104° F.; he was forced to quit his work; the epitrochlear mass had reached lemon-size by this time. He remained in bed for five days, during which time he experienced repeated chills and sweats, severe headache and backache. On November 21, he noticed for the first time a walnut-sized mass in the left axilla. A small, sharply punched-out ulcer was found at the site of the wound on the left middle finger on November 23. He returned to work on November 25, still feeling feverish and weak.

When examined by me on January 24, 1929, a small lemon-sized fluctuating epitrochlear mass was found, as well as a firm, nonfluctuant walnut-sized axillary mass. The primary lesion was almost completely healed. Serum collected at this time was found by George W. McCoy, of the Hygienic Laboratory, and by me, to agglutinate *Bacterium tularense* in all dilutions to 1:1280.

On January 31, 1929, partial excision of the epitrochlear mass was done. Approximately three ounces of thick yellowish exudate escaped. Microscopic examination of the tissue of the wall of the abscess showed multiple foci of caseous necrosis, surrounded by epithelioid and fibroblastic granulation tissue, containing many giant cells of the Langhans type, together with diffuse lymphocytic and polymorphonuclear infiltrations.

CASE 6.—Mr. F. C., patient of Dr. A. W. McCally, a twenty-eight-year-old factory worker, hunted rabbits near Lenoir City, Tennessee, on December 26, 1928. While cleaning several rabbits on that evening, he perforated the skin of the pad of the left thumb with

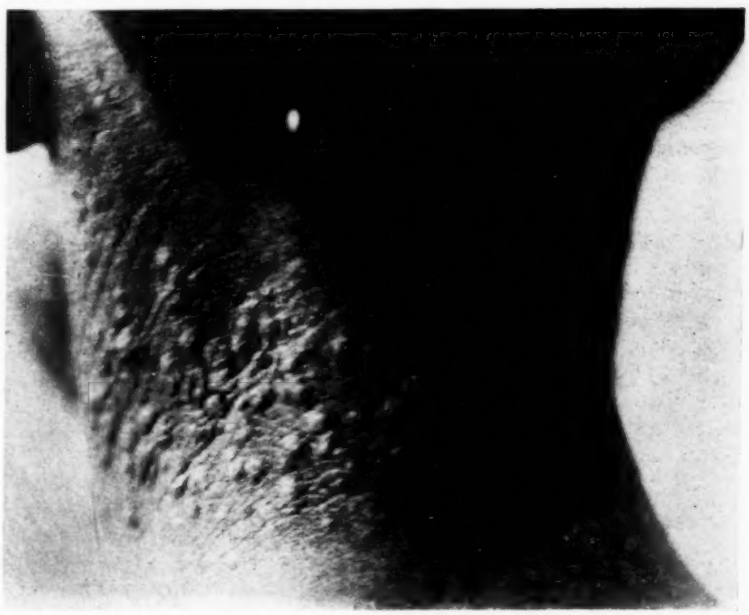


FIG. 2.—Mr. F. C., Case 6. Acneiform papular eruption of posterior cervical region. Appeared ten days after onset of illness. Disappeared in two months.

a sharp fragment of rabbit bone. Four days later, he experienced repeated chills of moderate intensity, accompanied by periods of feverishness and sweating. The extent of the fever is not known. On December 31, the patient complained of painful masses in the left epitrochlear region and in the left axilla. On January 1, he noticed for the first time a small ulcer of the left thumb at the site of the perforating wound. A Tennessee physician assured him that he had influenza. On January 7, the primary lesion was incised; no exudate escaped.

Mr. C. felt very weak for several days, but refused to take to his bed. Ten days after the onset of his symptoms, an acneiform papular eruption appeared over the back of the neck, over the dorsum of the hands and over the flexor surface of both forearms.

Mr. C. came to Dayton in search of work on January 15, 1929. He consulted Dr. McCally on January 19, who immediately arrived at the proper diagnosis. When examined by me on January 21, the eruption was unusually distinct (see Fig. 2). Firm walnut-sized masses were found in the left epitrochlear and axillary regions.

Serum of Mr. C., collected on January 21, 1929, was found by McCoy and me to promptly agglutinate *Bacterium tularense* in all dilutions to 1:1280.

When examined two months later, the eruption had cleared except for a few small lesions on the neck. The epitrochlear and axillary lymph nodes were of cherry-size, firm, and gave no evidence of suppuration.

CASE 7.—Mr. W. R., patient of Dr. O. C. Henderson, a forty-five-year-old proprietor of a fish and poultry market, skinned and dressed several cottontail rabbits on November 15, 1924. The rabbits had been shipped to Dayton from St. Louis, Missouri. While dressing the rabbits, Mr. R. scratched the middle finger of the left hand with a spicule of rabbit bone. On November 21, the left arm "felt hot and heavy," and he noticed a small egg-sized mass in the left axilla. The next day he noticed a small pea-sized papule on the left middle finger at the site of the scratch; the center of the papule sloughed out and left a small, deep, punched-out ulcer. Hot flax-seed poultices were applied to the axillary mass for three weeks, at the end of which time spontaneous evacuation of about two ounces of thick yellowish exudate occurred. Drainage persisted for over one month.

Because of the necessity of his being at his market each day during his illness, Mr. R. did not take to his bed, but was forced to sit quietly most of the time because of feverishness, chilliness, and marked weakness. Mr. R. did not undertake full-time work for three months. He knew at the time that he was a victim of "rabbit fever."

Serum of Mr. W. R., collected on May 16, 1929, four years and six months after the onset of illness, was found by McCoy and me to agglutinate *Bacterium tularense* in all dilutions to 1:160, with partial agglutination at 1:320.

CASE 8.—Mr. F. R., patient of Dr. O. C. Henderson, brother of Mr. W. R. (Case 7), a fifty-two-year-old electrician, was employed by his brother during the fall of 1916 as a meat-handler. Mr. F. R., while cleaning fish, the week before Thanksgiving, 1916, stuck a frozen fish fin under the nail of the right middle finger. He also perforated the skin of the tip of the fourth finger of the left hand in a similar manner on the same day. He did not actually dress rabbits on this day but did handle knives which other meat handlers had used in the cleaning of rabbits.

Two days after these injuries, both arms became swollen and painful with reddish purple streaks extending from the points of injury of both hands to the axillae. The axillary lymph nodes of both sides rapidly increased to lemon-size. On this day, the fever reached 104°, being initiated with a severe chill. This was followed by severe chills and sweats for three weeks, during which time Mr. R. was bedfast and experienced several periods of delirium. The first physician who was called made a diagnosis of pneumonia. A consultant made a diagnosis of infection of the fingers with blood poisoning. Multiple nodules developed along the lymphatics of both arms. The axillary nodules and those of the arms suppurated and were surgically incised. Drainage continued for four weeks. At the end of the third month a suppurating nodule appeared in the right posterior axillary fold. This was incised and drained for three weeks. Mr. R. was unable to do any work for six months. During the acute illness he lost ten pounds. He has been perfectly well since the end of the long convalescence. He emphatically avers that he has never touched a rabbit since this experience.

Serum of Mr. F. R., collected on May 31, 1929, twelve years and six months after onset of illness, was found by McCoy and me to agglutinate *Bacterium tularense* in all dilutions to 1:80—a titer which is in accord with that found in other long-recovered cases.

The following three cases, occurring outside of Dayton, were brought to my attention as the result of serologic studies carried out at the request of the physicians indicated. The clinical details are furnished through the courtesy of these physicians:

CASE 9.—Mr. H. H., patient of Dr. John Thomas Bowen, Clearwater, Florida, a sixty-nine-year-old retired business man, discovered a dead wild cottontail rabbit on the Belleview-Biltmore golf links on the morning of February 14, 1929. Influenced by the belief that the

possession of the left hind leg of a rabbit frequently improved one's luck, Mr. H. cut off the extremity. He observed that the animal was warm and flexible at the time of the dissection. During the amputation, Mr. H. broke a bone in the rabbit's leg and perforated the skin of his left thumb with the sharp end. He squeezed the thumb, causing it to bleed freely, sucked the wound for a few moments, and promptly dismissed the incident from his mind.

On the evening of February 16, some sixty hours after the episode described above, Mr. H. experienced chilliness, malaise, feverishness (102° F.), and headache. Physical examination by Dr. Bowen revealed an injected pharynx and a slightly enlarged spleen. The uranalysis showed a trace of albumin and occasional hyaline casts. A blood count showed 13,200 white blood cells.

On February 18, Mr. H. complained of painful swelling of the left thumb, and pain in the left axilla. Dr. Bowen discovered that the axillary lymph nodes were swollen to lemon-size. The left epitrochlear nodes were enlarged to walnut-size. In view of the suggestive history, Dr. Bowen suspected tularemia, while a surgical consultant insisted that the case was one of ordinary pyogenic infection. During the next week, the temperature varied between 101° and 103° F., with recurring chills.

Mrs. H. S., a lay member of the board of trustees of the Miami Valley Hospital, of Dayton, who was familiar with the work on tularemia which had been carried out in this laboratory, was wintering at the same hotel as the H. family. On February 25, Mrs. S. visited Mr. H. and expressed her conviction that he was suffering from tularemia.

On February 28, a macular and maculopapular eruption appeared over the face and upper anterior surface of the chest.

A long-distance telephone conversation with me strengthened the belief that Mr. H. was suffering from tularemia. At my request, blood specimens were submitted to the Hygienic Laboratory at Washington, D. C., and to this laboratory, where the serum of Mr. H., collected on March 4, 1929, was found to agglutinate *Bacterium tularense* in all dilutions to 1:1280.

The patient's temperature reached the normal level for the first time on March 7. The left axillary and epitrochlear lymph nodes remained firm and tender, and showed no evidence of suppuration up to the time of the last examination on March 18, 1929.

CASE 10.—Mr. C. C., patient of Dr. Franklin T. DuBois, Liberty, Indiana, a forty-year-old farmer, killed several rabbits during the early part of December, 1916. A few days after dressing the rabbits, a dime-sized, indolent, necrotic ulcer developed on his left thumb, at the site of a deep fissure in the skin, acquired while shucking corn some time before. The left axillary and epitrochlear lymph nodes became painfully enlarged at the same time. Mr. C. distinctly recalls chills, fever, sweats and backache. While he did not feel sick enough to remain in bed for any length of time, he was incapacitated for work for four months. The axillary adenopathy gradually subsided. The epitrochlear mass suppurated and was drained surgically; attempts to recover an organism on ordinary media from the exudate which escaped on incision were unavailing.

Serum of Mr. C., submitted to me on February 26, 1929, twelve years and two months after the onset of illness, was found to agglutinate *Bacterium tularense* in all dilutions to 1:80. This finding was confirmed by McCoy, at the Hygienic Laboratory.

CASE 11.—Mr. B. P., patient of Dr. Glen Nisley, Chillicothe, Ohio, a fifty-year-old meat handler, cut the palmar surface of the right index finger with a knife while dressing cottontail rabbits in a market during November, 1927. A few days after the injury, a deep ulcer developed at the site of the injury to the finger. A painful right axillary mass soon reached the size of a large orange. The development of the ulcer and adenopathy was simultaneous with the occurrence of recurring chills, high fever (105° F.), sweats, severe headache, backache and joint pains. The axillary mass suppurated and was surgically drained; a sinus tract persisted for several weeks. Mr. P. was unable to perform any work for eight weeks.

Serum of Mr. P., submitted to me on January 14, 1929, was found to give positive agglutination of *Bacterium tularense* in all dilutions to 1:160. Specimens submitted to Dr. R. E. Bower, Health Commissioner at Chillicothe, and to George McCoy, at the Hygienic Laboratory, gave identical results.

SUMMARY AND CONCLUSIONS

1. Recent advances in our knowledge of tularemia indicate that it is a world-wide disease.

2. Over 1000 cases have recently been discovered in the U.S.S.R. (Russia).

3. Over 800 cases have been reported during the past five years from forty states and the District of Columbia in this country.

4. Sixty-four proved cases have been investigated by me, sixty of which have occurred in one community—Dayton, Ohio.

5. While the wild rabbit continues to be the great reservoir of infection, many new animal hosts (wild rats and mice, sheep, muskrats, opossums, woodchucks, cats and game birds), and new insect vectors have been discovered during the past few years, thus pointing to the ever-widening dissemination of the disease among lower animal life and to new sources of infection for human beings.

REFERENCES

1. Francis, Edward, and Moore, Dunlop: Identity of Ohara's Disease and Tularemia, J. A. M. A. **86**: 1329, 1926.
2. Ledingham, J. C. G.: Some Observations on Tularemia, J. Path. & Bact. **26**: 132-133, 1923.
Ledingham, J. C. G., and Fraser, F. R.: Tularemia in Man from Laboratory Infection, Quart J. Med. **17**: 365-382, 1923-1924.
3. Nikanorov, S. M.: Tularemia in North America and Tularemia-like Disease in U. S. S. R., Rev. de Microbiol., d'Epidemiol. et de Parisitol. **7**: 289-293, 1928. (Russian text, English abstract.)
4. Suvorov, S. V., Wolferz, A. A., and Voronkova, M. M.: Plague-like Lymphadenitis in the Rayon of Astrakhan, Rev. de Microbiol., d'Epidemiol. et de Parisitol. **1**: 293-299, 1928. (Russian text, English abstract.)
5. Golov, D. A., Kniazevsky, A. N., Berdnikov, V. A., and Tiflov, V. E.: Plague-like Infection (Tularemia?) in the Region of the Basin of the Oural in the Spring of 1928, Rev. de Microbiol. d'Epidemiol. et de Parisitol. **7**: 301-326, 1928. (Russian text, French abstract.)
6. Dieter, L. V., and Rhodes, Bernice: Tularemia in Wild Rats, J. Infect. Dis. **38**: 541-546, 1926.
7. Perry, J. C.: Tularemia Among Meadow Mice *(Microtus californicus aestuarinus)* in California, Pub. Health Rep. **43**: 260-263, 1928.
8. Parker, R. R., and Dade, J. S.: Tularemia in Sheep in Nature, Pub. Health Rep. **44**: 126, 1929.
9. Green, R. G., Wade, E. M., and Dewey, E. T.: Experimental Tularemia in Muskrats, Proc. Soc. Exper. Biol. & Med. **26**: 426-427, 1929.
10. Schwartz, Harold: Tularemia From the Muskrat, J. A. M. A. **92**: 1180, 1929.
11. Mease, Jr., J. A.: Tularemia From Opossums, J. A. M. A. **92**: 1042, 1929.
12. Francis, Edward: Symptoms, Diagnosis and Pathology of Tularemia, J. A. M. A. **91**: 1155-1161, 1928.
13. McCoy, G. W.: Plague-like Disease of Rodents, Public Health Bull. **43**: 53, 1911.
14. McCoy, G. W., and Chapin, C. W.: *Bacterium tularense* the Cause of a Plague-like Disease of Rodents, Pub. Health Bull. **53**: 21, 1912.
15. Wherry, W. B., and Lamb, B. H.: Infection of Man With *Bacterium tularense* in Wild Rabbits, and the Danger of Its Transfer to Man, J. A. M. A. **63**: 2041, 1914.
16. Green, R. G., and Wade, E. M.: Tularemia in the Cat, Proc. Soc. Exper. Biol. & Med. **25**: 856-857, 1928.
17. Hanson, E. C., and Green, R. G.: Tularemia in Minnesota, J. A. M. A. **92**: 1920-1923, 1929.
18. Personal communication to the author.

19. Simpson, Walter M.: Tularemia: Study of Rapidly Fatal Case (Four Days, Seven Hours), Arch. Path. & Lab. Med. 6: 553-574, 1928.
20. Parker, R. R., and Spencer, R. R.: Tularemia and Its Occurrence in Montana, Montana State Board of Entomology, Sixth Biennial Report, pp. 30-41, 1925-1926.
21. Green, R. G., Wade, E. M., and Dewey, E. T.: Ruffed Grouse Are Susceptible to Tularemia, Proc. Soc. Exper. Biol. & Med. 25: 515-517, 1928.
22. Parker, R. R.: Quail as a Possible Source of Tularemia Infection in Man, Pub. Health Rep. 44: 999-1000, 1929.
23. Green, R. G., and Wade, E. M.: A Natural Infection of Quail by *Bacterium tularense*. (In press.)
24. Parker, R. R., Brooks, C. S., and Hadleigh, Marsh: The Occurrence of *Bacterium tularense* in the Wood Tick, *Dermacentor occidentalis*, in California, Pub. Health Rep. 44: 1299-1300, 1929.
25. Simpson, Walter M.: Tularemia (Francis' Disease). A Clinical and Pathological Study of Forty-Eight Nonfatal Cases and One Rapidly Fatal Case, With Autopsy, Occurring in Dayton, Ohio, Ann. Int. Med. 1: 1007-1060, 1928.
26. Simpson, Walter M.: Tularemia: Study of Rapidly Fatal Case (Four Days, Seven Hours), Arch. Path. & Lab. Med. 6: 553-574, 1928.
27. Simpson, Walter M.: Tularemia (Francis' Disease): Report of Four Additional Cases, Ohio State M. J. 24: 860-862, 1928.

For additional references see complete bibliography in Tularemia, by Walter M. Simpson, Paul B. Hoeber, New York, 1929.

DISCUSSION

Dr. J. C. Geiger.—The number of reported cases of tularemia in the medical literature have been considerably augmented by an interesting survey of Nevada by Doctor Meyer and myself of the George Williams Hooper Foundation for Medical Research of the University of California Medical School. Laboratory proof of 50 cases and epidemiologic evidence of 234 others with no deaths were submitted. All were of the gland and ulcer type with one exception, which was classified as typhoidal. The cases were stated to have been first observed in Nevada near Battle Mountain in 1912. This disease, having its bacterial origin discovered on the Pacific Coast and named accordingly, has been singularly absent or very few to be noted in the reports of these states. Perhaps, as this survey seems to indicate, the disease prevails to an unusual extent, at least in Nevada. Likewise, there appears to be every type of known transmission, rabbits, flies, ticks, contact with other animals apparently healthy such as dogs, coyotes, and sheep and such extraneous material as knife blades and barbed wire. Moreover, one proved case was attributed to bites of mosquito though the report admits this information was most vague. The question of immunity of the Indians in Nevada, who use rabbits as sources of meat, to tularemia has been settled since these investigators submit laboratory proof of one case in a Piute and record of others in the Shoshones.

Dr. W. T. Cummins.—I enjoyed very much the presentation of this paper and the discussion of Dr. Greiger. In the Southern Pacific General Hospital, San Francisco, in a period of thirty days, we had seven tularemic patients from Nevada. Dr. Geiger rendered very valuable assistance along the Southern Pacific Lines in calling attention to the menace of tularemia. Our seven cases were apparently the result of three rabbit contacts, three tick bites, and one fly bite. They made uneventful but protracted recoveries.

Dr. C. W. Bonynge.—We have just recently found a case of tularemia in a cattleman from Utah. The mode of infection was not definitely determined, but it was felt that it came through an insect bite. This man had an intense preauricular and submaxillary adenitis. An interesting point which might be brought out in these cases, is that the literature leads one to believe cross agglutination between *Bacterium tularense* and *Brucella abortus* and *melitensis* is quite frequent. Francis states that this was true in 25 per cent of his cases, and in several the titer was practically identical with all three organisms.

Our case of tularemia shows *no* agglutination with *Brucella* and in our *abortus* work, cross agglutination with melitensis is very rare. I do not believe cross agglutination between tularemia and *Brucella abortus* can be depended upon.

Dr. Warren T. Vaughan.—I want to ask Dr. Simpson in regard to the long drawn out cases, whether he suspects that there may be a chronic form, or whether there is a pos-

sibility of a carrier state? Is there involvement to the central nervous system? Some of the sequelae which he has described suggest that there may have been a central nervous invasion.

Dr. Robert F. E. Stier.—I can truthfully say that we in the Northwest have been on the lookout not only for undulant fever but for tularemia. We found one suggestive case. This patient was only in Spokane as a patient. His home was in British Columbia. The only thing we can say of it was that the histologic picture of the large mass in the axilla was suggestive of tularemia. I have been trying to get some blood from that patient ever since then but have been unable to. So far as Spokane is concerned I have been on the lookout for it. I have some of Dr. Francis' antigen and also have sent some blood back to Dr. Francis for checking. In three or four tests that were compared the results were all negative. It is possible that a diagnosis of typhoid is being made in the acute cases, so when the general practitioner does not recognize these conditions the laboratory man has nothing to go by.

Dr. Frederic E. Sondern.—Might we ask Dr. Simpson to say a few words relative to safeguarding of the laboratory workers.

Dr. Walter M. Simpson (closing).—It is true that only four cases of tularemia have been reported from California. This is of unusual interest in view of the fact that the disease was first discovered in the ground squirrels of Tulare County in that state. None of the cases of tularemia in human beings has been traced to contact with the ground squirrel. The ground squirrel tick is apparently a feeble transmitter of the infection.

As regards the cross-agglutination of *Bacterium tularense* and the *Brucella*, Francis and Evans found that 37 of the sera from 100 cases of tularemia in the human being showed cross-agglutination of *Brucella abortus* and *Brucella melitensis*. In only three instances were the antitularense and antiabortus-melitensis agglutinins identical. Of the remaining 63 sera, many of which were of high antitularense titer, there was no cross-agglutination.

The history of contact with infected rodents, flies, and ticks and the proportionately higher titer reached by antitularense agglutinins usually leaves no doubt as to the proper interpretation of the serologic findings. In the event of cross-agglutination in those rare instances in which the agglutinin titer of all three organisms is the same or nearly the same, agglutinin absorption tests may be employed. As regards the serum-agglutinins in tularemia, all investigators agree on these four points: first, that agglutinins do not develop until after the first week of illness; second, that agglutinins once acquired never disappear from the serum, making it possible to determine whether or not a person has had the disease at some time in the past; third, that subsequent exposure to the infection does not elevate the declining trend of the titer; fourth, that one attack confers a permanent immunity.

In answer to Doctor Vaughan's question, I would state that in the prolonged cases in which the patient has been incapacitated for many months, the acute illness lasted from two to three weeks. No one has found the organism in the blood after the twelfth day. The convalescence in these cases is characterized by extreme prostration. I do not believe that there is any actual central nervous system involvement, but that the nervousness, restlessness and insomnia are associated with the general debility. There is no evidence that the individual suffers from repeated recurrences of the disease.

It is hazardous to make a diagnosis of tularemia from tissue sections alone. Many tissue pathologists have confused the granulomatous lesions of tularemia with those of tuberculosis.

A RECENTLY ISOLATED BACILLUS OF THE HEMOPHILIC GROUP*

By F. W. Hartman, M.D., and Edna Jackson, M.S., Detroit, Michigan

THE recent epidemic of influenza again sharpens our interest in the etiologic factor or factors in this disease and also in Pfeiffer's bacillus which was described in 1892 and was considered the most probable organism concerned at least up until the 1918-1919 epidemic. One of the facts which demonstrates Pfeiffer's bacillus to be pathogenic for man is the production of meningitis with a 10 per cent mortality as shown in the 82 cases reviewed by Torrey.[1] Anderson and Schultz[2] collected 94 cases occurring between the 1889-1890 epidemic and the 1918-1919 epidemic.

When a small gram-negative coccobacillus, aerobic and hemophilic, was isolated from a ten-year-old child in the service of Dr. J. C. Montgomery, a provisional report of Pfeiffer's bacillus was made while further cultural studies and animal work were done.

ISOLATION OF ORGANISM

The patient was a white male child ten years of age. A history of chickenpox shortly before the onset of the present illness two and one-half weeks previously was given. The present illness began with weakness, drowsiness, and impaired strength in the right leg and foot. On admission the patient was drowsy and irritable and there was slight rigidity of the neck. Kernig's sign was positive and there was papilledema in both eyes. Temperature ranged from 101 to 104° F. Laboratory examinations showed, blood: hemoglobin 11.3 gm. per 100 c.c., erythrocytes 4,584,000, leucocytes 7,400, polymorphonuclears 74; small mononuclears 22; large mononuclears 4. Urine: albumin +; sugar 0; hyaline and granular casts ++. Spinal fluid, slightly turbid; distinct pellicle formed in twelve hours; leucocytes 90 per cm. all lymphocytes; sugar 36 mg. per 100 c.c. Culture: small gram-negative coccobacillus. The organism obtained from the spinal fluid of this case could not be distinguished in morphology from B. influenzae. Its cultural and morphologic characteristics are described below:

Morphology.—The organism, referred to in this article as culture 155, occurs as a small slender bacillus 1-2 microns in length, but more commonly as a small coccobacillus 0.6 to 0.8 microns in diameter. It is nonmotile, capsules have not been demonstrated and spores are not formed. A few longer forms, 2 to 5 microns, have been seen, but these are not common.

Staining Reactions.—The organism is stained by the usual aniline dyes. It is gram-negative. When stained with Giemsa's, the center of the bacillus stains more deeply than the extremities.

*Read before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.

From the Laboratories of the Henry Ford Hospital.

Cultivation.—The organism is distinctly aerobic, and when first isolated could be grown only upon media enriched by blood or yeast extract.

Best growth is obtained on rabbit blood agar. On this media a good growth occurs in from eighteen to twenty-four hours. The colonies are moist, translucent, mucoid in appearance but only slightly viscid in consistency. There is a marked tendency for the growth to become confluent, isolated colonies occurring only at the edges of the growth on an agar slant, or on plates where the inoculation was sparse. The isolated colonies are often irregular in outline, and may attain a diameter of 2 to 3 mm. Maximum growth occurs in forty-eight hours, after this the growth flattens down and the mucoid appearance becomes less marked. No hemolysis occurs.

Dextrose ascitic agar cultures generally produce a fair growth, although this is much less profuse and less constant than that on blood agar. The growth is moist and the colonies confluent.

Plain agar cultures were successful only after the organism had been carried on artificial media for three months. The growth on plain agar is very scant, being scarcely visible after two days. Yet fifteen successive transplants were carried out on plain agar. Smears made from plain agar generally show the small bacillus forms.

Dextrose hormone broth is evenly clouded and there is no pellicle formation. Smears made from this media show small coccobacilli more frequently than the slender bacillus form.

Fermentation Reactions.—Sugar fermentation tests were made in tubes of Dunham's peptone broth to which were added 0.1 c.c. of yeast extract and 1 c.c. of 10 per cent solution of the sugars to be tested. Acid is produced in dextrose, levulose, galactose, mannite, saccharose and xylose. No acid is formed in maltose or lactose. Litmus milk is not changed.

Indol tests have usually been positive. Nitrates are reduced to nitrites. Gelatin is not liquefied. Tests for production of amylase as described by Rivers[3] are negative.

Toxicity.—Rabbits were inoculated intravenously with 5 and 10 c.c. of (1) saline suspensions of the organism killed by heating at 56° one hour, (2) seven-day broth cultures killed by heating at 56° one hour, and (3) seven-day broth cultures which had been filtered through a Berkefeld filter and tested for sterility. None of the rabbits showed any toxic effects.

PATHOGENICITY

In all 45 animals, 2 guinea pigs, 3 monkeys, 17 rabbits, and 23 dogs, were used. In these the organism was almost invariably fatal for all except the monkeys which resisted large doses intravenously, intratracheally and intracerebrally. It must be said however that the organism had lost much of its virulence for other animals before the monkeys could be obtained. The pathology produced is best studied with reference to the site of inoculation.

Of the two rabbits and four dogs inoculated intracerebrally, both rabbits and three dogs developed meningitis and the gram-negative bacillus was seen

in direct smears and recovered in cultures in pure form. The fourth dog was semicomatose for three days and then improved, but gradually lost weight and was sacrificed on the eighteenth day. Brain was negative on gross exami-

Fig. 1.—Lungs from dog one week after intratracheal injection of 10 c.c. of twenty-four hour broth culture of gram-negative bacillus. Both lobes on the left and the lower lobe on the right are consolidated. Small abscess cavities stud the pleural surface. On section much greyish red viscid purulent material exudes from the surface.

Fig. 2.—Lungs from dog forty-eight hours after intravenous injection of 7½ c.c. twenty-four hour broth culture of gram-negative bacillus. The lobes are voluminous and firmly consolidated. On section the parenchyma is dark red in color having the general appearance of blood clot.

nation and culture. The lower lobe of the left lung was partially consolidated and a hemolytic streptococcus was recovered in pure culture. The gram-negative bacillus was not recovered although the course was similar to that of other animals that survived the first few days.

The course in the meningitic animals varied from one to four days. All were drowsy or semicomatose and showed neck rigidity. The gross lesions were characterized by localized or general purulent and often hemorrhagic exudates over the cortex extending about the pons and medulla. Microscopically there was edema and swelling of the meninges and in most cases hemorrhage throughout. Of the cellular infiltration leucocytes and eosinophiles predominate with a good proportion of round and wandering cells. The brain substance was not involved but in some areas there is a marked perivascular infiltration.

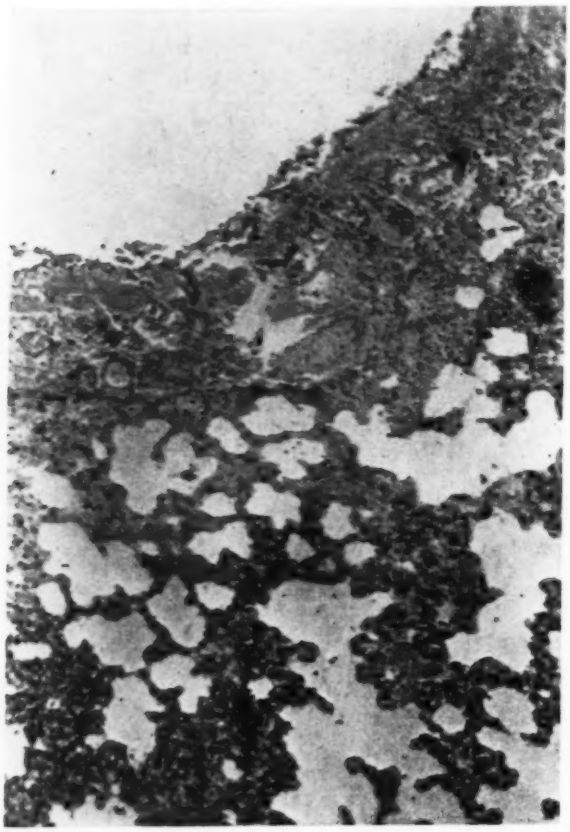


Fig. 3.—Low power through wall of bronchus and parenchyma. The lining epithelium is denuded and the adjacent tissue necrotic taking a uniform red color as contrasted with the tissue further away which shows much emphysema and hemorrhage.

One rabbit and one guinea pig, each inoculated intraperitoneally, showed a diffuse peritonitis, in the pig the reaction was hemorrhagic, in the rabbit more purulent. The gram-negative bacillus was recovered in both instances in pure culture.

One guinea pig inoculated subcutaneously developed an abscess at the site of inoculation with the surrounding tissue infiltrated by blood.

The thirteen rabbits and five dogs inoculated intravenously (dosage ranged from 1 to 2.75 c.c. for rabbits and up to 10 c.c. for dogs) showed localization

principally in the joints, lungs, pleural, and peritoneal cavities. The rabbits were more prone to joint lesions while the dogs with one exception showed more marked lesions in the lungs. The spleen was enlarged and congested as were also the liver and kidneys but only exceptionally were abscesses or hemorrhages found in these organs. Diffuse hemorrhagic lesions including the intestine were seen twice.

The joint lesions were characterized by collections of grayish-yellow or hemorrhagic exudate in the bursae and joint cavity. Erosion of joint surfaces was not observed.

Fig. 4.—Low power lung parenchyma including two bronchioles after introtracheal inoculation. The lining of the bronchioles is denuded and the parenchyma is infiltrated with blood.

The peritoneal exudates were usually thick, viscid and stringy but in some cases were comparatively thin and only slightly turbid. On the other hand the pleural involvement was more often hemorrhagic both as regards the surface and the exudate. In a few instances both visceral and parietal pleura and the pericardium were thickened and hemorrhagic.

The lungs were similar in the animals inoculated intravenously and intratracheally as far as gross anatomy is concerned. The intratracheal dosage ranged up to 10 c.c. and was administered during morphine narcosis and by

means of a De Vilbiss spray. The lungs of animals succumbing in the first three days showed consolidation of one or more lobes with areas of consolidation in other lobes. (Figs. 1 and 2.) Often all lobes were solid and hemorrhagic throughout. The visceral pleura was smeared with the hemorrhagic pleural exudate. The glands about the hilus were enlarged, soft and dark red on section. The trachea and larger bronchi showed slight congestion and never ulceration. The smaller bronchi were filled with thick, viscid, grayish-red exudate. The cut surface of the early lesions was dark red and firm but contained some air. In later lesions the cut surface was softer, grayish-red in color and oozed

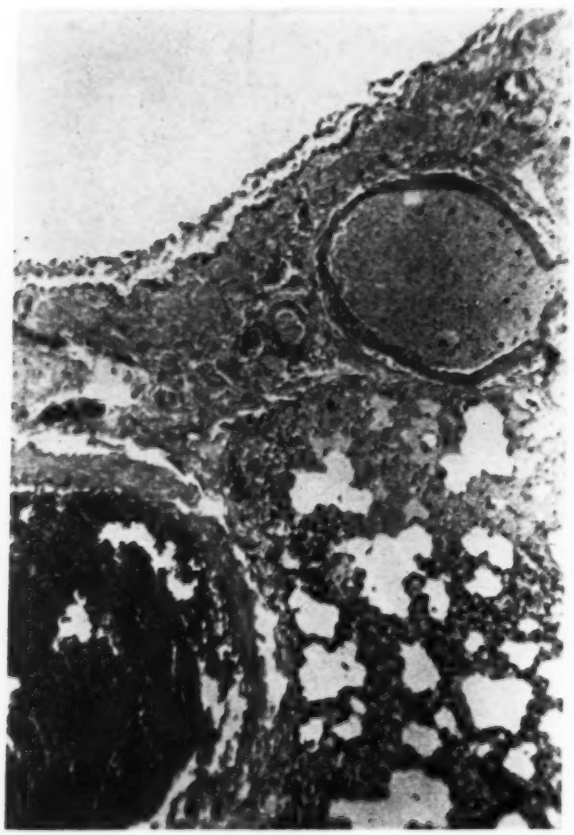


Fig. 5.—Low power lung parenchyma through wall of bronchus; the lining epithelium is denuded and the adjacent parenchyma is necrotic. Farther away are enlarged blood vessels, and hemorrhagic emphysematous parenchyma is seen.

thick, viscid, grayish purulent material. Animals living two weeks or more often showed small abscesses throughout all lobes. These were filled with thick, grayish-red, viscid, purulent material and were not well walled off.

Microscopically there are no certain differential points to determine site of inoculation, but the lesions vary considerably according to age. The early lesions are characterized by exudation in the small bronchi and bronchioles and often desquamation or necrosis of the lining epithelium. (Figs. 3 and 4.) The

alveoli adjacent may contain fluid or blood or both, but the alveolar walls are thickened and infiltrated by round, wandering cells and leucocytes. The blood vessels were engorged, and in many cases the walls of the smaller vessels are infiltrated with blood while there is a collar of blood immediately about the vessel. In lesions of three days' standing or more the alveoli are filled with leucocytes and still later definite abscess formation may occur with or without retention to the bronchi. Emphysema is a prominent feature of the earlier lesion with dilated and confluent alveoli scattered abundantly and diffusely through the lung. (Fig. 5.)

Since the morphology and the growth requirements of culture 155, when first isolated, suggested that the organism might belong to the group of influenza bacilli, three cultures of this organism and four strains of B. influenzae were compared in regard to some of their growth requirements. Three of the cultures of B. influenzae were obtained from Parke, Davis & Company, one No. 211 from the Detroit Board of Health Laboratories. The latter had been recently isolated from the spinal fluid in a case of influenzal meningitis.

The autoclaved blood was prepared by adding sterile rabbit's blood (5 per cent) to hormone broth. This was autoclaved for twenty minutes at 20 pounds, filtered, tubed, and sterilized at 15 pounds for thirty minutes. To each one-half of the tubes, 0.1 c.c. of yeast extract was added. All cultures were kept ten days before being called negative. The results are shown in Table I.

TABLE I

COMPARISON OF GROWTH REQUIREMENTS OF CULTURE 155 AND FOUR CULTURES OF B. INFLUENZAE

	155	155-1	155-2	B. INFLUENZAE			
				01768	01835	01291	211
Hormone broth	slight	slight	slight	–	–	–	–
Hormone broth plus whole rabbit's blood	+	+	+	+	+	+	+
Hormone broth plus autoclaved blood	+	+	+	–	–	–	–
Hormone broth plus autoclaved blood plus yeast	+	+	+	+	+	+	+
Dex. asc. agar	slight	slight	slight	–	–	–	–
Plain agar	very slight	very slight	very slight	–	–	–	–

From these results it is evident that this organism is much less strict in its growth requirements than the true influenza bacillus, since it will grow upon media containing only the thermostabile factor in blood, or upon media enriched by ascitic fluid or yeast, even showing slight growth upon plain agar.

Since this organism bears some resemblance to bacilli of the Pasteurella group, and since cases of human infection with members of this group have been reported (Mayer and Hoppli[4]), a comparison was made with four strains of Pasteurella cuniculicida. Three of these stains were obtained from Parke, Davis & Company, one, No. 131, from the American Type Culture Collection. These four strains agreed in fermenting maltose and not fermenting xylose,

thus differing from the fermentation reactions of the organism discussed here. None of these four cultures showed the translucent growth on blood agar that has been so characteristic in the cultures of this organism, yet that characteristic might be possessed by some strains. Moreover the bipolar staining characteristic of the Pasteurella group has never been observed in the study of this organism either in smears from cultures or in smears from tissue of inoculated animals.

TABLE II

COMPARISON OF CULTURAL REACTIONS OF CULTURE 155, AND TWO STRAINS OF PASTEURELLA CANICULICIDA

CULTURE	155	01315	131
Dextrose	A	A	A
Levulose	A	A	A
Galactose	A	A	A
Mannite	A	A	A
Maltose	–	A	A
Lactose	–	–	–
Saccharose	A	A	A
Xylose	A	–	–
Gel. (liq.)	–	–	–
Indol	±	+	+
Nitrates	+	+	+

From time to time various gram-negative bacilli have been reported which bear a marked resemblance to Pfeiffer's bacillus in morphology and manner of growth, but which cannot be classified as belonging to that group because of differing growth requirements. Among these there might be mentioned Hemophilus para influenzae,[5] described by Rivers, Hemophilus canis, originally isolated by Friedberger,[6] and Bacillus meningitidis cerebrospinalis septicemiae isolated by Cohen[7] from three cases of meningitis.

Hemophilus canis resembles the organism which we have described here in its fermentation reactions, in nitrate reduction, but differs from it in its opaque growth on blood agar, and in its lack of pathogenicity for rabbits and guinea pigs. Also it is apparently more strict in its growth requirements.

Cohen's organism has much in common with culture 155, in that it is essentially hemophilic yet can be grown upon dextrose ascitic agar; it shows a translucent confluent growth upon blood agar; it occurs as small bacillus similar to Pfeiffer's, also as small coccobacillus, especially in broth; it stains more deeply at the center than at the extremities, and in animals it produces a true septicemia.

From these studies it would seem that this organism isolated from a case of meningitis must belong to a group of organisms essentially hemophilic, yet not as strict in their growth requirements as either the true influenza bacillus or the para influenza bacillus described by Rivers. Therefore, if this organism is isolated in additional cases and continued observation confirms it as a distinct species, the designation Hemophilus para influenzae B. is proposed.

SUMMARY

1. An essentially hemophilic organism isolated from a case of a fatal meningitis is described.

2. Morphologically this organism cannot be distinguished from various members of the hemophilus group.

3. Culturally this organism resembles the Pasteurella group more closely than the hemophilus group but the fermentation reactions show essential differences.

4. Pathogenicity of the organism described is much more marked for laboratory animals, including dogs, than organism of either the hemophilus or Pasteurella group compared with it, the most striking and constant lesion being a hemorrhagic interstitial pneumonia.

5. Freshly isolated gram-negative coccobacilli should be subjected to exhaustive cultural work and animal inoculation before classification is made.

REFERENCES

1. Torrey, Robert G.: Influenzal Meningitis With a Report of a Case, Am. J. Med. Sc. **152**: 403-410, 1916.
2. Anderson, R. A., and Schultz, O. T.: Immunologic Study of Strains of Bacillus Pfeifferi Isolated From a Case of Meningitis, J. Exper. Med. **33**: 653-666, 1921.
3. Rivers, T. M.: The Biological Classification of Influenza Bacilli, Johns Hopkins Hosp. Bull. **31**: 50-53, 1920.
4. Mayer, M., and Hoppli, R.: Ueber eine menschliche Infektion met einem Bakterium aus der Gruppe der Hämorrhagischen Septikämie (Pasteurellose), Centralbl. f. Bakt. **90**: 241-246, 1923.
5. Rivers, T. M.: Influenza-Like Bacilli; Growth of Influenza-Like Bacilli on Media Containing Only an Auto-clave-labile Substance as an Accessory Food Factor, Bull. Johns Hop. Hosp. **33**: 429-431, 1922.
6. Freidberger, E.: Ueber ein neues zur Gruppe des Influenzabacillus gehöriges hämoglobinophiles Bakterium (Bacillus haemoglobinophilus canis), Centralbl. f. Bakt. **33**: 401-406, 1903.
7. Cohen: La méningite cérébro-spinale septicémique, L'Inst. Pasteur Ann. **23**: 273-311, 1909.

HENRY FORD HOSPITAL.

EMBRYONAL CARCINOMA OF THE TESTICLE*

By L. W. Larson, M.D., Bismarck, North Dakota

A DESIRE to report six cases of embryonal carcinoma of the testicle and incidentally to review the voluminous literature on this subject has led to the preparation of this paper.

FREQUENCY

Testicular tumors are comparatively rare. Tanner[1] found that approximately 600 cases had been reported in the literature previous to 1922; that in 110,000 male admissions to the London Hospital, 65 had tumors of the testicle; that in 300,000 admissions, both male and female, to the Mayo Clinic, there were about 50 testicular tumors; and, that about one in 2000 male admissions (0.05 per cent) to a general hospital has this serious condition. Hinman[2] states that in 182,729 general male hospital admissions, there were 116 cases (0.063 per cent) of testicular tumor, and that there had been 649 cases reported in the literature. Morton[3] found that 102 patients who had a tumor of the testicle had been operated upon up to the end of 1927, at the Mayo Clinic. The mortality statistics of the United States Bureau of Vital Statistics show that malignant tumors of the testicle constitute about 0.6 per cent of all malignant tumors in men.

HISTORY

A tumor of the testis was described in 1696 by St. Donat[4] in which a rudimentary skull and the embryonic eyes of a parasitic fetus were found. Johnson,[4] in 1856, was the first to ascribe a tridermal origin to certain tumors of the testicle. Langhans and Kocher,[4] in 1887, with the aid of the microscope, ventured the opinion that most of the testicular tumors are teratomas.

ORIGIN

Ewing,[5] in 1911, after having reviewed the literature thoroughly and having analyzed 19 cases, came to the conclusion that practically all testicular tumors are teratomatous in origin; that in those cases in which one type of tissue predominates, apparently at times to the exclusion of all other types of tissue, the true explanation lies in the assumption that the predominant tissue represents an overgrowth of that particular derivative, and the tumor, although teratomatous in origin, assumes the appearance of a unicellular type of tumor.

Chevassu,[6] in a classic thesis, showed that 59 out of 120 cases of testicular tumor were of a solid medullary large celled type. He stressed the resemblance of these cells to the spermatocyte, and, assuming that they were

*Read at the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.

From the Department of Pathology, Quain and Ramstad Clinic, Bismarck, North Dakota.

derived from the cells of the seminal tubules, called the tumors "seminomes." The term "seminome" or "spermatocytoma" has since been used by Debernardi,[7] Nicholson,[8] Tanner,[1] Schultz and Eisendrath,[9] Southam and Linnell[10] in describing this particular type of tumor.

Ewing has remained steadfast in his belief that these tumors are teratomas mainly because he has often seen the characteristic appearing "seminome" cells in teratomas, and, in very early embryonal carcinoma, he has found minute traces of cartilage, entodermal alveoli, and squamous epithelial cell groups. So, Ewing[11] is forced "to conclude that this common tumor of the testis is always a one-sided development of a teratoma, and is not derived from the adult spermatoblasts." Hinman, et al., who have written voluminously on the subject, and who favored the monodermal theory of origin, in a recent publication[12] report that they have found tissues of various types associated with typical seminomatous tissue, and conclude that "the term 'seminome' or 'spermatocytoma' must, therefore, be regarded as a misnomer, and the contention of Chevassu is disproved in favor of Ewing's theory." In a Hunterian Lecture, Cairns[13] substantiates the views of Ewing. Morris[14] found typical "seminome" cells in a metastatic nodule in the lung from a case of teratoma of the testis and agrees with Ewing in that practically all tumors are essentially teratomas. The evidence, therefore, seems fairly strong in favor of the theory that practically all testicular tumors arise from totipotent sex cells and are of teratomatous origin. The so-called seminome is a modification of the carcinomatous degeneration found in most testicular tumors.

Recently, Stevens and Ewing[15] have reported a case of adenocarcinoma of the testis, which they believe arose from the testicular tubules. They state that Gordon Bell had previously reported four such cases, in three of which Ewing concurs in the diagnosis. This type of tumor is characterized by an occurrence in a later decade of life, and a slow growing affair with a relatively good prognosis. It would appear, therefore, that there are two varieties of testicular carcinomas, the common type of embryonal carcinoma and the rare adenocarcinoma.

I believe that it is fairly well agreed (Ewing,[4] Hinman[12]) that primary sarcoma of the testicle is comparatively rare. Dew[16] believes that only about 2 per cent of testicular tumors can be regarded as primary sarcomas. Undoubtedly, a large proportion of the sarcomas reported in the literature is, in reality, carcinomas.

CLASSIFICATION

Granting, therefore, that all testicular tumors are teratomas, Ewing's[17] classification seems to be quite acceptable, especially to the clinician. He divides them into three main varieties:

1. Adult embryoma, or teratoma.
2. Embryoid, teratoid, or mixed tumors.
3. Embryonal malignant tumors.

The adult embryomas constitute a very small group of testicular tumors and are similar to the dermoids found in the ovary. The majority of them appear to be congenital, Cairns[13] finding an 84 per cent occurrence during the

first year of life in a series of 47 cases. They are usually benign, some having been known to exist for forty years, and simple orchectomy produces a cure.

Embryoid, teratoid or mixed tumors comprise about one-half of the malignant testicular neoplasms. They are composed of undifferentiated embryonal tissue, derived from all three germ layers, and so arranged that any resemblance to a fetus is lacking.

These tumors tend to appear during the third decade. They are moderate or large in size and may be solid or contain cysts. Cut section presents a variety of appearance, depending on the proportion of cysts and tissue elements present, arrangement, etc. The cysts are lined by squamous or cylindrical cells, and the solid portions consist of embryonal connective tissue, smooth muscle, cartilage, bone, etc. The malignancy of the tumor depends on the tendency of derivatives of one germ layer to outgrow and overgrow the others.

In 1902, Schlagenhaufer[18] reported the presence of chorionic elements in a teratoma. Subsequent writers have verified this observation. (Handfield-Jones,[19] Morton,[20] etc.)

Tumors with tissue of this type occur either alone or in a teratoma. The chorionic elements are identical in structure to those found in women. They are very malignant and the primary growth frequently is so small that it escapes detection until the patient comes complaining of symptoms due to metastases. It is possible that a number of the primary hepatic chorionic tumors reported have been metastatic tumors from an overlooked chorioma of the testis.

Cases have been reported (Ewing quoting Warthin,[11] Cairns[13]) in which hypertrophy of the breasts and even colostrum secretion have been noted in patients with teratoma of the testicle. Since these tumors contain the essential structures of a new individual, and since apparently the presence of this new individual stimulates the breasts to activity, it must be admitted that a pseudogestation in the male is within the realm of possibility.

EMBRYONAL TUMORS OF THE TESTIS

Into this group fall the seminome of Chevassu, a majority of the tumors reported in the literature as sarcoma, embryonal carcinoma, etc. It comprises slightly more than half of the malignant tumors of the testicle.

Grossly, this tumor consists of a perfectly homogeneous white or greyish white tissue, which is divided into lobules by fine and coarse fibrous strands. Areas of necrosis are almost constant, and small points of hemorrhage are frequent. The tumor is usually large, but may be small and scirrhous.

The microscopic appearance of these tumors is usually characteristic. The cells are large and spheroidal, with nuclei of a powdered appearance. The stroma tends to be lymphoid in type and lymph nodules may form. The resemblance of these cells to sarcoma cells is apparent and explains the wide variation in nomenclature found in the literature. It is over this group of tumors that the controversy as to origin discussed above centers, Chevassu and his followers claiming that they are derived from the cells of the seminal

tubules, and Ewing maintaining that they are merely one-sided developments of a preexisting teratoma. Whatever their point of origin, it is agreed that they constitute a distinct type of testicular tumor, both as to structure and malignancy.

ETIOLOGY OF TERATOMA TESTIS

Relation to Injury.—In spite of the fact that most men have injured their testes at some time in their lives and relatively few develop neoplasms of these organs, a surprising number of patients presenting themselves with such tumors give a definite history of a previous injury. Cairns[13] found 14 out of 79 patients (18 per cent); Tanner[1] found 22 in 100 (22 per cent); 3 of our 6 patients (50 per cent) gave a definite history of injury. Chevassu[6] states that traumatism favors the growth of testicular tumors, but believes that this theory has been overrated.

Relation to Position of the Testicle.—The majority of writers agree that the undescended testicle is more prone to develop a teratoma than the normal organ. Aurousseau[22] after summarizing 76 cases calculates that 10 per cent of the malignant tumors affect the abnormally placed testis, and since only one out of a thousand testicles is found outside the scrotum, the relative incidence of tumor in the undescended testicle is greater than in the normal organ. Lund[23] says that malignant tumors of the normal testis are 10.3 times as frequent as in the testis in the abnormal position, but draws attention to the fact that only one in five hundred testes is abnormally placed. He quotes Bowing who states that malignancies of the undescended testicle constitute one-third of testicular malignancies. Tanner[1] concludes that testes within the canal are more apt to become malignant. Cairns[13] believes that the tendency of the undescended testicle to become malignant is overemphasized, and quotes Howard, who after a wide experience has come to the same conclusion. Two of our 6 cases (33 per cent) were in undescended testes.

Age.—Most testicular tumors occur during the ages of sexual activity. Tanner[1] found one under five years, none between six and seventeen years, 42 between eighteen and twenty-nine years, and 32 between twenty-nine and thirty-nine years. These tumors are comparatively rare in children. Only 5 of Chevassu's[6] 61 cases of teratomas occurred in children under five years of age, and none of his 59 seminomes. Philipp collected 42 cases of testicular tumors in children from the literature up to 1908 (quoted by Kutzman[21]). Kelley (quoted by Kutzman[21]) states that a few dermoids have been reported in children. Our case of embryonal carcinoma in a child one year of age is probably the first to be reported. Kutzman and Gibson[21] find only one case of seminome in children reported in the literature, and that in a child seven years of age.

Testicle Involved.—Cases reported in the literature show that the right and left testicles develop tumors with about equal frequency. Two of our 6 cases were in the right testicle and 4 in the left testicle.

Bilateral Occurrence.—This is an extremely rare phenomenon, Chevassu[6] finding only one in 128 patients.

METASTASES

All teratomas, whether mixed type or embryonal, are malignant and eventually metastasize. Extension is first along the spermatic lymphatics and veins. Secondary tumors in the lumbar nodes are common, Cairns[13] reporting 29 out of 33 cases. An epigastric tumor involving the nodes of the celiac axis is often the first sign of metastasis. Metastases may extend up into the mediastinal and cervical nodes, where they may reach great size. Cases are on record in which the tumor spread continuously by means of the veins up to the heart. Embolic nodules in the lungs, liver, brain, kidneys, and stomach are more common as a result of venous passage. Invasion of the vertebrae with remittent paraplegia occasionally occurs. Likewise, involvements of one or both ureters followed by hydronephrosis and uremia have been reported.

DIAGNOSIS

It is not within the province of this paper to discuss the clinical diagnosis of testicular tumors. However, the diagnosis is primarily a matter of exclusion. In the differential diagnosis, inflammatory conditions, hydrocele, spermatocele, tuberculosis, gumma, and tumor must be considered.

PROGNOSIS

Malignant testicular tumors are recognized as among the most malignant neoplasms known. Chevassu[6] reports 19 per cent living at the end of four years among 100 patients. Tanner[1] reports 465 followed cases: 377 or 81 per cent were dead from metastases, and 25 or 5.5 per cent were living and well four years or more after orchectomy. He found that the mixed type of teratoma is more malignant (90 per cent) than the embryonal type (60 per cent).

The prognosis appears to be especially unfavorable in children. Kober (quoted by Kutzman[21]) found that 4 out of 10 patients died of metastases within one year of castration, one was living two months postoperative, and the other five had not been heard from. Steffen (quoted by Kutzman[21]) after reviewing 25 cases of his own and from the literature found that 13 patients were dead from recurrence or metastases within eleven months after operation, 7 were living, and of these 7, only 2 were well, and 7 were not heard from; therefore, of his followed cases, the mortality was 80 per cent.

TREATMENT

Several methods of treatment have been advocated. Simple orchectomy will cure those cases in which metastasis has not occurred, but it is impossible to always detect the presence of early metastasis into the nodes usually involved. Since simple orchectomy gives four-year cures in only 10 to 15 per cent of the cases, a more drastic treatment has been advocated by Hinman and others. In 1914, Hinman[24] reported a collection of 46 cases in which a radical operation had been performed. This procedure removes the lymph zones in the retroperitoneal and lumbar areas along the aorta and vena cava. The operative mortality was 11 per cent. Forty-six per cent were alive, 1 five years, 1 four years, 5 three years, 2 two years, and 11 one year or less following operation. There was a probable cure in at least 4 patients who

had invasion of the lumbar nodes at the time of operation. In a later publication Hinman[25] reports a total of 79 cases in which a 30 per cent cure resulted from the radical procedure. Cairns[13] states that out of 74 cases reported from the London Hospital, 55 of the patients had simple orchectomy and 19 had the radical operation. There was no operative mortality. Recovery after orchectomy was 33 per cent, and following the radical operation it was 31.2 per cent. This might be accounted for by the fact that those

Fig. 1.—Case 1. Gross specimen.

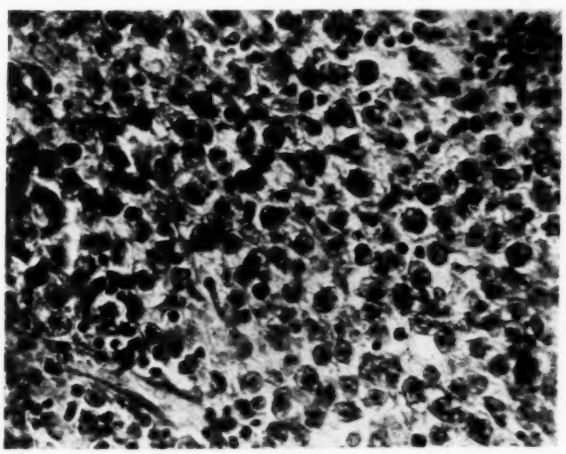


Fig. 2.—Case 1. Photomicrograph.

patients in whom the radical operation was performed had mostly mixed tumors and therefore of greater malignancy than the single type cell tumors that comprised the majority of the patients given simple orchectomy.

Coley[26] reports 78 cases of malignant tumors of the testicle treated with a combined vaccine of Bacillus prodigiosus and Streptococcus erysipeloides, in which 22 patients or 28 per cent survived more than two years.

Numerous writers recommend radium and x-ray therapy, either alone or as an adjunct to surgery. Dean[27] reports 9 out of 13 patients with primary

operable tumors in which orchectomy was not done, living from six months to five years one month after radium and deep x-ray therapy; 4 out of 16 patients with primary inoperable tumors with metastases living from six months to five years five months; all three patients with local recurrences after orchectomy living from three years to ten years; 26 out of 81 patients (33 per cent) with inoperable recurrences and metastases following orchectomy living from three months to nine years three months, 9 of whom lived

Fig. 3.—Case 2. Gross specimen.

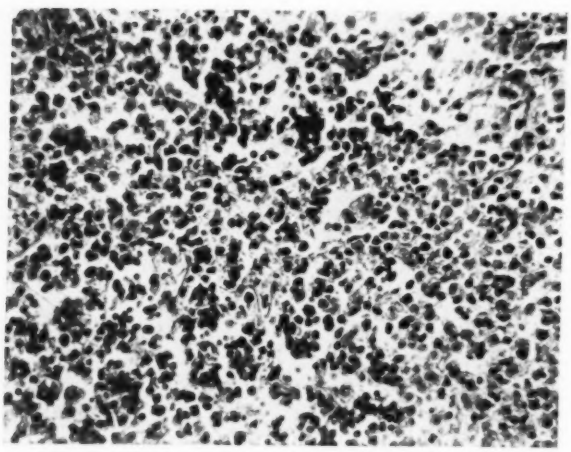


Fig. 4.—Case 2. Photomicrograph.

over two years; and 8 out of 11 patients (72 per cent) who received prophylactic treatment after orchectomy living from six months to nine years. Reports such as these tend to prove that radium and x-ray therapy have not only a palliative value, but probably vie with surgery in the average case. The embryonal types are more radiosensitive than the mixed teratomatous types.

Ewing, in a personal communication last year, doubted the value of the radical removal of regional lymph nodes and recommended the primary use

of x-ray therapy, to be followed by orchectomy and then more x-ray therapy. Higgins[28] advocates the same procedure.

Our procedure has been the use of x-ray therapy following orchectomy, which I believe is also followed at the Mayo Clinic.

CASE REPORTS

CASE 1.—Henry W., aged thirty-eight, German, farmer, married. Came to our Clinic May 25, 1921, because of an undescended left testicle and a tumor in the left inguinal region. Past history was negative. Present illness began eight weeks ago when tumor mass in left

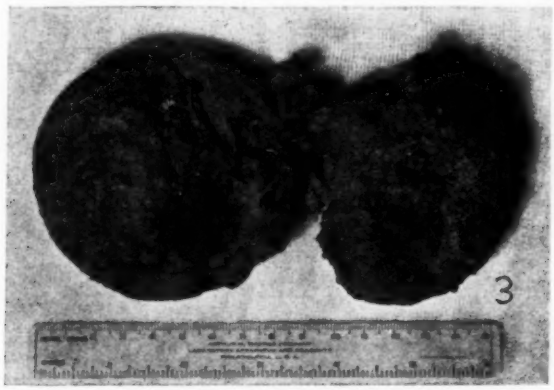


Fig. 5.—Case 3. Gross specimen.

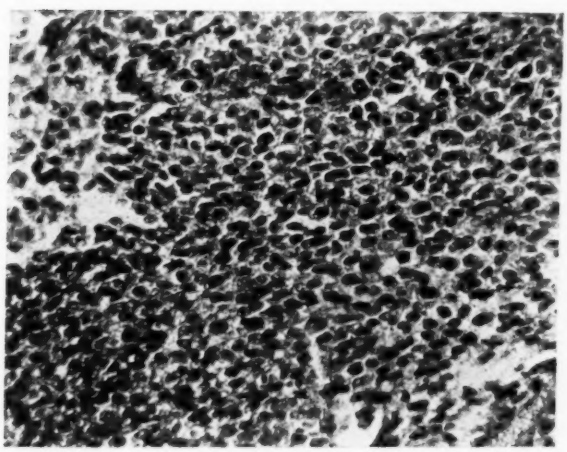


Fig. 6.—Case 3. Photomicrograph.

inguinal region began to appear. The tumor had grown progressively and was so painful that he could not work.

Physical examination revealed an undescended left testicle and a large tumor in the left inguinal region. The general physical examination was negative. No evidence of metastasis could be demonstrated. Orchectomy was done May 28, 1921.

The specimen consisted of a well encapsulated soft tumor which, on cut section, presented a pale pink solid surface. The epididymis was not involved. Paraffin sections revealed an embryonal carcinoma in a lymphoid stroma. No other tissue elements could be recognized.

He was given light x-ray therapy to four areas in the left inguinal region on June 30, 1921. A letter from the patient stated that he was well on May 28, 1929, eight years after operation.

CASE 2.—James R., aged thirty-nine, Russian, farmer, married. Came to our Clinic March 4, 1925, complaining of a swollen left testicle. Past history was negative. Present illness began ten months ago when he was kicked by a horse. The left testicle was injured and began to swell very soon after the accident. There was pain in the testicle for some time after the injury, but lately the pain has subsided. The testicle has grown progressively larger since the injury. No other complaints.

Physical examination revealed a robust well-appearing male. Except for an advanced

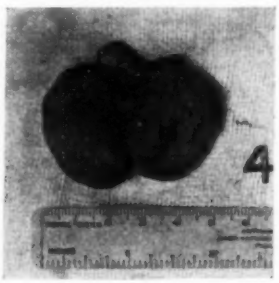


Fig. 7.—Case 4. Gross specimen.

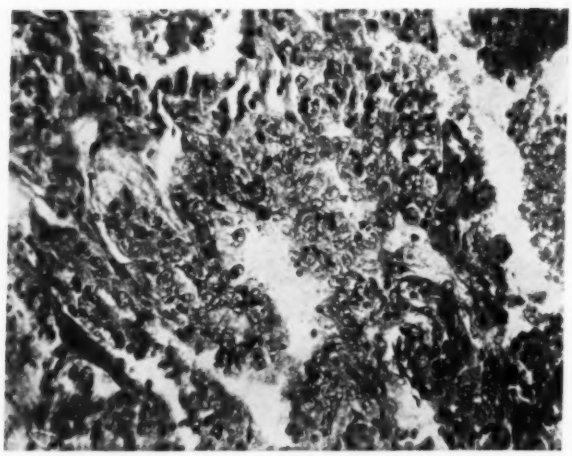


Fig. 8.—Case 4. Photomicrograph.

pyorrhea and several infected teeth, the general physical examination was negative. The left testicle was enlarged to about twice the normal size, and was firm and smooth. Orchectomy was performed March 5, 1925.

The specimen consisted of a large well encapsulated tumor measuring 5 by 6 by 8 cm. The testicular tissue could not be recognized grossly. The epididymis appeared uninvolved. Cut section presented a spongy, lobulated, yellowish white surface of similar character throughout.

Paraffin sections revealed the typical embryonal type of carcinoma in a lymphoid stroma. No other tissues could be recognized.

The patient was given deep roentgen therapy to the testis, groin and mediastinum, beginning March 9, 1925, and seven times thereafter at irregular intervals until May 27, 1926.

From January until June, 1926, he complained of much abdominal and left kidney pain. He died September 3, 1926, eighteen months after operation.

CASE 3.—Joe C. L., aged thirty, single, laborer. Came to our Clinic March 13, 1925, complaining of backache and a rupture. Past history was negative except that he had always had a left-sided rupture. No history of injury. The present illness began three weeks ago with backache and pain in the left side. There was no fever nor chills. Since the pain began the rupture had been "out" and did not go back again.

Examination showed negative findings except for a questionable left inguinal hernia, and a left hydrocele.

Fig. 9.—Case 5. Gross specimen.

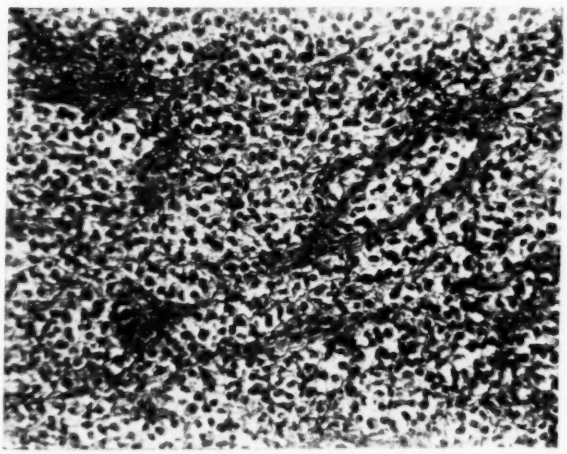


Fig. 10.—Case 5. Photomicrograph.

Operation was performed March 16, 1925, and about an ounce of straw colored fluid was found in the left tunica. The testicle was enlarged to the size of an orange. Orchectomy was done and a left inguinal hernia repaired.

The specimen consisted of an egg-shaped tumor measuring 5 by 6 by 9 cm. All testicular tissue appeared to be destroyed. The tumor was well encapsulated and on cut section presented a lobulated grey surface with small areas of hemorrhage and degeneration. The epididymis was not involved.

Paraffin section revealed an embryonal carcinoma in a lymphoid stroma. No other tissues could be recognized.

He was given deep roentgen therapy, anterior and posterior, to the lower abdomen and left groin, on March 25, 1925. He left the hospital April 4, 1925, and returned April 27th complaining of pain in the left renal area. At this time he was given deep x-ray therapy to the lower abdomen and also to the left renal area. The patient left for his home in Illinois in May, 1925. He entered a hospital there, but was not given x-ray treatment. A letter from his physician stated that he died November 1, 1925, six and one-half months after operation.

CASE 4.—Arnold W., aged one year, was brought to us June 4, 1926 because of a progressive increase in the size of the left testicle since birth. History negative. Normal birth. Weight 9 pounds at birth. Breast fed. Had done well.

Fig. 11.—Case 6. Gross specimen.

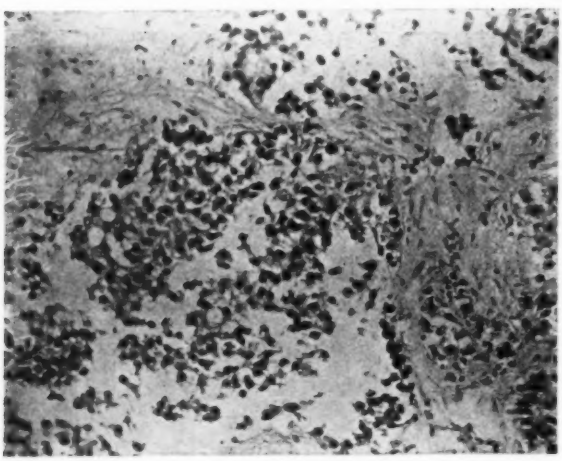


Fig. 12.—Case 6. Photomicrograph.

Examination revealed signs of rickets. A hard tense tumor about the size of a small hen's egg was present in the left side of the scrotum. It did not transilluminate light. No areas of softening or fluctuation could be detected.

Orchectomy was done June 5, 1926.

The specimen measured 2½ by 3 by 4 cm. The entire testicular substance was replaced by a solid pearl colored tumor which was well encapsulated. There were no areas of softening or necrosis. One small area appeared to be hemorrhagic. The epididymis was not involved.

Paraffin sections revealed cords and nests of embryonal carcinomatous tissue in a lymphoid stroma. No other tissue elements could be recognized.

No x-ray or radium treatment was given. A letter from the father stated that the child was living and well June 5, 1929, three years after operation.

CASE 5.—Richard P., aged thirty, American, miner, married. Consulted our Clinic on January 14, 1929 complaining of a lump in the right groin. First noticed a painless lump about the size of an almond in the right groin in 1917. A year ago the lump began to increase in size. Three months ago he slipped and strained the right limb and from then on the lump slowly increased in size. Last week following a strain while lifting, the lump increased in size to that of two large thumbs. Had never had pain in the lump until the past week and since then it has pained him only on exertion. The past history was essentially negative.

Physical examination was negative except for a freely movable, painless mass the size of a small potato in the right groin. The right testicle was not in the scrotum. The left testicle was normal.

Operation was performed January 15, 1929. A tumor was found in the right inguinal region lying just below the external inguinal ring. The mass removed by the multitherm electrocautery knife and the hernia repaired.

The specimen measured 2½ by 5 by 8½ cm. The enlargement was symmetrical, the surface was smooth, and the epididymis was normal. The capsule was smooth and intact. The corpus was firm and somewhat nodular and on cut section presented a lobulated, moist, greyish white surface on which numerous fine connective tissues could be seen. There was no gross evidence of degeneration nor could any normal testicular tissue be recognized.

Paraffin sections showed a typical embryonal carcinoma in a lymphoid stroma. No other tissue elements could be recognized.

He was given a course of x-ray therapy on January 24, 1929. He was living and well on June 15, 1929, six months after operation.

CASE 6.—Godliph R., aged twenty-four, German, single, farmer. Consulted our Clinic on March 16, 1929 complaining of an enlargement of the right testicle. He first noticed the enlargement seven months ago. Has no pain in the testicle except on manipulation. Has doubled in size during the past six weeks. No other complaints. Past history revealed lung trouble for a year, nine years ago. Had a cough, hemoptysis, etc., but did not consult a physician, so he does not know whether he had tuberculosis or pneumonia. Has also had much trouble with pes planus.

Physical examination showed a well developed and well nourished young male. There was a submaxillary and cervical adenopathy present, a compensated mitral insufficiency, intact inguinal rings, pes planus, and a firm smooth enlargement of the right testicle. The lungs were negative.

Orchectomy was performed March 18, 1929.

The specimen measured 3½ by 4 by 7 cm. It was very firm and its capsule was smooth and vascular. The epididymis was smooth and normal, grossly. Cut section through the corpus showed almost the total testicular substance, with the exception of a small area in the superior portion at the periphery, replaced by a greyish white diffuse tumor which had a moist surface.

Paraffin sections showed an embryonal carcinoma with numerous strands of fine connective tissue in which a few lymphocytes could be found.

A series of x-ray treatments were given from March 27 to 29, 1929, and a second series on April 30, 1929. He was living and well on June 18, 1929, three months after operation.

SUMMARY OF CASES REPORTED

An analysis of our cases reveals some interesting observations (see Figs. 13 and 14). The ages of the patients ranged from one year to thirty-nine years, four occurring during the third decade, and one during the second decade, and one in infancy. Three (50 per cent) patients gave a definite history of a previous injury to the organ. The length of time that the patients

had been conscious of an abnormality varied from three weeks to ten or twelve months. Two of the patients complained of constant pain in the testicle, two experienced pain only when the organ was handled or in the least way traumatized, one had had pain but was free from pain when he consulted us, and the infant had never complained of pain. Four occurred in the left testicle and two in the right testicle. In three cases (50 per cent) a hydrocele complicated the tumor. Two cases (33 per cent) were in undescended testes and a like number were associated with an inguinal hernia, in one case (Case 5) both the abnormal position of the testicle and an accompanying hernia being present.

CASE	AGE	DURATION	PAIN	TRAUMA	TESTICLE INVOLVED
1	38	8 wk.	+	–	L
2	39	10 mo.	Pain after trauma—subsided	Kicked by horse 10 mo. ago	L
3	30	3 wk.	+	–	L
4	1	10-12 mo.	–	–	L
5	30	Lump groin 12 yr. Increased size 3 mo. ago	On exertion only	In childhood; also 3 mo. ago	R
6	24	7 mo.	Only on manipulation	While riding horse	R

Fig. 13.—Analysis of case reports

CASE	HERNIA	TESTICLE UNDESCENDED	WITH HYDROCELE	X-RAY THERAPY	RESULT
1	–	+	+	+	Living 8 yr.
2	–	–	+	+++ Large doses	Died 18 mo.
3	+	–	+	++	Died 6½ mo.
4	–	–	–	–	Living 3 yr.
5	+	+	–	+	Living 5 mo.
6	–	–	–	+	Living 3 mo.

Fig. 14.—Analysis of case reports.

The mortality to date has been 33 per cent, one patient living six and one-half months and one patient eighteen months after orchectomy. In both of the fatal cases, the patients obtained intensive x-ray therapy. The patient that has lived the longest (eight years) received only two sittings of small doses of roentgen therapy, while the infant, who has lived three years, received no x-ray treatment. Cases 5 and 6 have received massive doses of x-ray therapy, but they are both such recent cases that conclusions could not be fairly drawn from them. Thus, a measure of the efficacy of x-ray treatment from a study of our cases cannot be determined. The only logical conclusion must be that in the fatal cases, metastases had taken place by the time operation was performed, while in the apparently cured cases (Cases 1 and 4) the neoplasm was limited to the testicle.

COMMENT

Because of the nature of the organ from which tumors of the testicle arise, it seems reasonable to suppose that most of them would have a complex structure. The evidence set forth by Ewing, Hinman, and others seems

conclusive that they are all essentially teratomatous. We have been unable to find any other derivatives than the embryonal type of carcinomatous cells in our primary tumors. However, we have not had the opportunity of making postmortem examinations of any of our cases and thereby determine the type of tissue found in all the metastatic nodules. The important consideration regardless of varying opinions as to tissue derivatives, classifications, nomenclature, etc., is that the type of tumor herein reported constitutes a distinct clinical entity with a greater radiosensitiveness and, therefore, a better prognosis than the tumor that is obviously teratoid in structure.

The question of the etiology of tumors is always an interesting one. All that we have been able to suggest with regard to testicular neoplasms are contributing factors only. Just what influence trauma, position of the testicle, previous infections, etc., have on the subsequent development of a neoplasm, it is most difficult to evaluate from the data at hand. Certainly, age must be an important factor since most of the testicular tumors develop between the ages of eighteen and forty years. This is the period of greatest sexual activity in the average male and it seems reasonable to suppose that this factor is a very important predisposing one. And yet, the occurrence of a tumor in a child one year of age, such as the one herein reported, which is similar to the type of tumors occurring in mature men, would appear to entirely overthrow the theory that sexual activity is the most important contributing factor. Not until the causative agent or agents are discovered will we be able to properly evaluate the meager information we have as to etiology.

REFERENCES

1. Tanner, C. O.: Surg. Gynec. Obst. **35**: 565-572, 1922.
2. Quoted by Cabot, Hugh: Modern Urology, ed. 2, 2 vols., Philadelphia and New York, 1924, Lea & Febiger.
3. Morton, S. A.: Proceedings of the Staff Meetings of the Mayo Clinic **4**: 98-100, 1929.
4. Quoted by Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, 1928, W. B. Saunders Co.
5. Ewing, J.: Surg. Gynec. Obst. **12**: 230, 1911.
6. Chevassu, Maurice: Tumors du testicle, Paris, 1906, No. 193, p. 239.
7. Debernardi, Larenzo: Beitr. z. path. Anat. u. z. allg. Path. **40**: 534-600, 1907.
8. Nicholson, G. W.: Guy's Hosp. Report **61**: 249-321, 1907.
9. Schultz, O. T., and Eisendrath, D. N.: Arch. Surg. **2**: 493-520, 1921.
10. Southam, A. H., and Linell, E. A.: Brit. J. Surg. **11**: 223-233, 1923.
11. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia and London, W. B. Saunders Company, p. 840.
12. Hinman, Frank, Gibson, Thomas E., and Kutzman, Adolph A.: Ann. Surg. **82**: 552-575, 1925.
13. Cairns, H. W. B.: Lancet **1**: 845-850, 1926.
14. Morris, J. H.: Arch. Surg. **15**: 530-541, 1927.
15. Stevens, A. R., and Ewing, James: Ann. Surg. **88**: 1074-1079, 1928.
16. Dew, Harold: Surg. Gynec. Obst. **46**: 447-458, 1928.
17. Ewing, James: Neoplastic Diseases, ed. 3, Philadelphia and London, W. B. Saunders Company.
18. Schlagenhaufer, F.: Wien. klin. Wchnschr. **15**: 571-580, 1902.
19. Handfield-Jones, R. M.: Brit. Surg. J. **13**: 606-620, 1926.
20. Morton, S. A.: Proc. Staff Meetings Mayo Clinic **3**: 77-79, 1928.
21. Kutzman, A. A., and Gibson, T. E.: Ann. Surg. **78**: 761-784, 1923.
22. Aurousseau, L.: Jour. de Chir. **27**: 17-30, 1926.
23. Lund, Fred B.: Boston M. & S. J. **190**: 533-536, 1924.
24. Hinman, Frank: J. A. M. A. **63**: 2009-2015, 1914.
25. Hinman, Frank: Surg. Gynec. Obst. **37**: 429-451, 1923.
26. Coley, W. B.: Ann. Surg. **78**: 370-386, 1923.
27. Dean, A. L., Jr.: Am. J. Surg., N. S. **5**: 32-38, 1928.
28. Higgins, C. C.: Ann. Surg. **87**: 276, 1928.

DISCUSSION

Dr. Charles R. Drake.—Tissue pathology is of special interest to me, and this particular type of pathology has been more or less confusing. I feel that we must be careful and not be too dogmatic in our terminology, so that the more I have studied these types of tumors the more I have been inclined to call them "malignant tumors of the testicle" rather than to classify them in any particular way. Wherever there are epithelial cells, there also you can have carcinoma no matter where it is. As to the two groups of pictures of Dr. Larson's, they were individually more or less uniform in character. One group showed the variation in type of growth and emphasized the fact of not being too dogmatic about terminology. Epithelial cells may change from the normal, and when the epithelial cells become malignant, they do not continue in their normal type of growth. Not only do they change in the methods of growth but also in morphology and therefore can take on multiple types of shape and character as shown by these slides. A year or two ago I saw a tumor of the testicle about 5 cm. in diameter which was typically adenocarcinoma. These are somewhat rare. There was no suspicion of any other type of growth in it. This was confirmed by other clinics. All these tumors are malignant or nearly so. I cannot help but feel like emphasizing the necessity in not being too dogmatic in our classification of these tumors and that a carcinoma may originate in any part of the body, at any time, from any source, wherever there is epithelial structure.

Dr. Zera E. Bolin.—I have personally seen quite a number of Dr. Hinman's slides and those of Dr. Rusk. I think that I probably have seen two or three tumors which were cut up to small pieces and practically entirely blocked and nothing could be found but an adenocarcinomatous arrangement. It is interesting to note that the author found a large number of rest cells embryonic in type. It might be that these are responsible for some of the tumors.

Dr. Roy W. Hammack.—There is just one point which I wish to bring up: that is the question of the relation of trauma to etiology as it comes up under the Workmen's Compensation Act. Of course, the whole subject of the relation of trauma to tumors comes up there also. Everyone can recall an injury at some time or other to the testicle, and the workmen who develop malignant tumors are no exception. When these cases come before the Industrial Accident Commissions they must attempt to settle the question. Now, it seems to me that the literature is indefinite on the subject as I suppose it must be. I have just reviewed fourteen or fifteen papers published in the last few years, and I think there were only four in which this question was discussed. All of these rather tended to decry the idea that trauma is a factor of importance. When these cases do come before the Industrial Accident Commissions, the pathologist is usually called upon to express an opinion either by the insurance company in defense, or else by the beneficiary. What is he going to say?

Dr. Leonard W. Larson.—I think that Dr. Drake's remarks about being conservative in the classification of testicular tumors is well taken. I do not wish to give the impression that this particular type of testicular tumor is the only type found. We have had five cases since I came to Bismarck, and I find record of one before that time, making a total of six. This, of course, is merely a coincidence, and it shows that reporting a small series of cases does not mean so much. However, it is interesting to note that we have seen this type of tumor in six instances during a five-year period. If you will investigate the article written by Hinman recently you will find a classification of testicular tumors that covers about four pages. I think Ewing's attempt to put them into a few simple types is a very good one.

I was much interested to hear Dr. Bolin's statement that he had seen a pure adenocarcinoma. I am sure Dr. Ewing would be much interested in seeing Dr. Bolin's case because when I talked to him over a year ago he was very enthusiastic because he had found such a case. He has reported that case within the last month or so.

The relation of trauma to the etiology of tumors is a very important one. I do not know what the medical profession is going to do about it. We see a great number of cases in which this question comes up because the Workmen's Compensation Bureau is located in our city. Recently, an elevator operator, who had fallen and injured his chest came in with

an abscess of the chest which had developed at the point of injury some time after the accident. On examination we found that he had an old tuberculosis in both lungs and that the abscess was a tuberculous abscess. Of course he and his friends are going to prove to the Workmen's Compensation Bureau that the injury caused the abscess. There should be some way of getting together on these things.

Dr. O. A. Brines.—There is no use saying this matter has all been cleared up. It is still somewhat confusing, and yet I think that the whole thing has been to quite a large extent clarified, and I think that the idea that we are dealing primarily with mixed tumors is becoming gradually accepted. The most difficult thing is to hit upon a real good name to call them; probably one is as good as the other. Teratoma is all right except that they do not resemble the average teratoma. It certainly is true that the epithelial tissue in these neoplasms is the important thing. The other tissue does not really enter in very much. The greatest plea that can be made is to simplify the present classification, and instead of the large number of terms now being used to describe these tumors, perhaps one name can be used for all of them.

OXALIC ACID AS A REAGENT FOR ISOLATING TUBERCLE BACILLI AND A STUDY OF THE GROWTH OF ACID-FAST NONPATHOGENS ON DIFFERENT MEDIUMS WITH THEIR REACTION TO CHEMICAL REAGENTS*

By H. J. Corper, M.D., Ph.D., and Nao Uyei, Ph.D., Denver, Colo.

IN DETERMINING the value and limitations of a method for isolating tubercle bacilli from contaminated tuberculous materials for diagnostic purposes, consideration must be given to many factors which may contribute to the simplicity and accuracy of the procedure for practical purposes. Earlier studies by us,[1] reported before the American Society of Clinical Pathologists as part of the outline of the Research Committee, have established that an appropriate culture method could be used advantageously in place of guinea pig inoculation for the diagnosis of tuberculosis when the bacilli were not found to be present in the ordinary stained smear examined microscopically.[2] The method essentially consists in destroying the contaminating microorganisms in the tuberculous material by the addition of an equal volume of 6 per cent sulphuric acid and after thorough mixing, incubating for thirty minutes, diluting the acid with sterile saline and planting the sediment on a 6 per cent glycerol water crystal violet potato cylinder medium.[3] This medium was found in comparative quantitative tests to be the best nutrient medium, of all those tested, for the purpose of supporting the growth of tubercle bacilli when present in only small numbers in the tuberculous materials, as well as being best suited, due to the crystal violet present, to suppress the growth of such rapidly growing microorganisms as may have survived the sulphuric acid treatment.

There are certain undesirable features to the use of sulphuric acid, such as its marked avidity for water which makes likely an alteration in concentration after long standing, especially in strong stock solution. Its corrosive action on the hands and clothing is also a disadvantage to its use. In order, if possible, to find a reagent not possessing these undesirable features, a survey of other acids was made with the result that oxalic acid, obtainable in pure crystalline form and, therefore, of definite chemical composition, proved encouraging in that it gave promising results in preliminary tests which indicated a higher yield of cultures as well as a lower percentage of contamination.

I. A COMPARISON OF OXALIC ACID AND SULPHURIC ACID AS A REAGENT FOR DESTROYING CONTAMINATORS

In an effort, therefore, to improve and simplify the culture method for tubercle bacilli as far as possible the following experiments were performed in elaboration of earlier encouraging preliminary tests. For the purpose of

*Read at the Eighth Annual Convention of the American Society of Clinical Pathologists, at Portland, Oregon, July 5, 6, and 8, 1929.

From the Research Department, National Jewish Hospital at Denver, Colorado.

comparing the efficiency of oxalic acid as compared to sulphuric acid sixteen positive tuberculous sputums were obtained and after thorough mixing were divided into one cubic centimeter portions, placed in 15 c.c. graduated centrifuge tubes, each specimen treated with an equal volume of different concentrations of oxalic acid, from saturated to 2 per cent solution, and for the purpose of control a similar one cubic centimeter specimen was treated in the usual prescribed manner with an equal volume of 6 per cent sulphuric acid. After incubating the well mixed acid treated specimens for thirty minutes at 37° C., with occasional shaking during this time, each was diluted by the addition of 10 c.c. of sterile 0.9 per cent saline solution and after centrifugation the sediment was planted on tubes of crystal violet potato cylinder medium and read after regular intervals of incubation at 37° C. The findings resulting from this comparative study are recorded in Table I.

TABLE I

A COMPARISON OF OXALIC ACID AND SULPHURIC ACID AS REAGENT IN THE TREATMENT OF POSITIVE TUBERCULOUS SPUTUMS FOR ISOLATING TUBERCLE BACILLI

REAGENT USED	CULTURE RESULTS IN PER CENT*	
	POSITIVE TUBERCLE BACILLUS ISOLATIONS†	CONTAMINATIONS
6 per cent Sulphuric Acid	78	28
Saturated Oxalic Acid (about 9.5 per cent)‡	86	15
7 per cent Oxalic Acid	85	14
5 per cent Oxalic Acid	88	15
2 per cent Oxalic Acid	66	45

*The percentage results were calculated from the number of culture tubes presenting positive cultures of tubercle bacilli or contaminations as compared to the total number of tubes used in each test. There were five tubes planted from each sputum in each case, making a total of eighty tubes for each reagent.

†On the basis of sputums alone the results were 100 per cent in that every sputum gave at least one positive culture for tubercle bacilli even including those treated with the 2 per cent oxalic acid reagent.

‡International Critical Tables published for the National Research Council by McGraw-Hill Book Company 4: 250, 1928, gives 9.52% oxalic acid ($C_2H_2O_4.2H_2O$) soluble at 20° C.

It is to be noted from an examination of the results recorded in Table I that the findings with saturated or 7 or 5 per cent oxalic acid were almost identical so far as percentage isolations of pure cultures and percentage contaminations were concerned, while 2 per cent oxalic acid proved to be decidedly less serviceable both in destroying contaminators as well as providing pure cultures of tubercle bacilli. In concentrations of 5 per cent to a saturated solution oxalic acid also proved to be more satisfactory than 6 per cent sulphuric acid, in that a definitely lower percentage contaminations resulted from its use as well as a greater percentage of positive cultures of tubercle bacilli being yielded.

The foregoing results with positive sputums suggested a greater germicidal action of the oxalic acid in 5 per cent to saturated solution (equal volume being added) toward the contaminators as well as a reduced toxicity toward the tubercle bacilli as compared with an equal volume of 6 per cent sulphuric acid in sputums. It, therefore, appeared advisable to determine further whether any difference existed in control experiments with seedings with pure suspensions of tubercle bacilli in tuberculostatic tests. For this

purpose tubercle bacilli were planted in graded amounts upon tubes of crystal violet potato medium to which had been added one cubic centimeter of 6 per cent glycerol water containing varying amounts of oxalic acid from 0.0001 per cent to 5 per cent. The seedings with finely divided suspensions of virulent human tubercle bacilli ("Gluckson") ranged from 0.000,001 to 1.0 mg. The growth on this medium at 37° C. using glycerol water crystal violet potato cylinder medium as control was read at regular intervals and recorded. The results after eight weeks incubation are recorded in Table II.

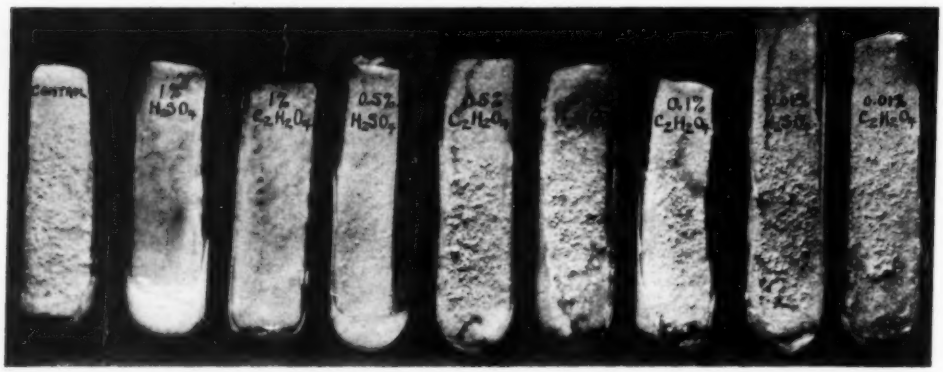


Fig. 1.—Tuberculostatic action of oxalic acid ($C_2H_2O_4$) in glycerol water crystal violet potato cylinder medium compared with that of sulphuric acid (H_2SO_4). The medium was planted with a suspension of virulent human tubercle bacilli (Gluckson) containing one milligram per cubic centimeter and incubated for three weeks at 37° C. The concentration of oxalic or sulphuric acid in the glycerol water added to the crystal violet potato cylinder is designated for each tube in the photograph. Note the decided inhibition of growth with the 0.5 per cent sulphuric acid which was hardly appreciable with the 0.5 per cent oxalic acid at this time.

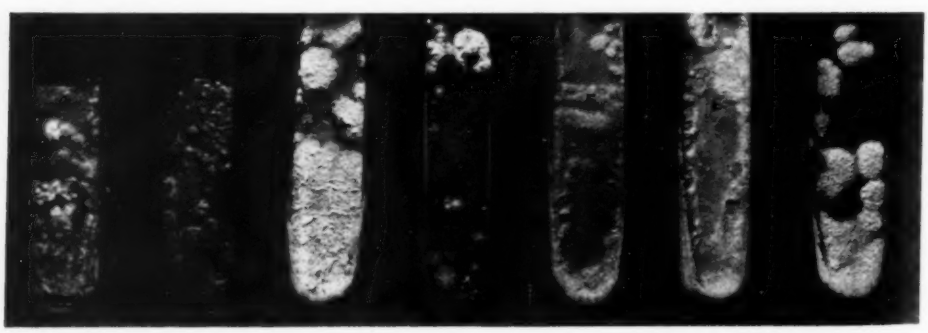


Fig. 2.—Growth of smegma bacilli on different mediums planted with a suspension containing 0.000,1 mg. per c.c. and incubated fourteen days at 37° C. Note growth on all the mediums slightly less so on Long's nonprotein agar medium. From left to right the tubes of medium are (1) glycerol water potato cylinder medium; (2) glycerol water crystal violet potato cylinder medium; (3) medium "P" (25 per cent ground potato, 2½ per cent glycerol and 1½ per cent agar); (4) Long's nonprotein medium; (5) Petroff's gentian violet egg medium; (6) Dorset's egg medium, and (7) 5 per cent glycerol broth agar.

It is clearly evident from the findings recorded in Table II that oxalic acid in 0.1 per cent concentration in the glycerol water exerts no tuberculostatic action, while concentrations of 1 per cent or more exercise a definite retarding influence upon the growth of the tubercle bacilli on the crystal violet potato medium. Now if we apply this information to determine whether or not the oxalic acid used to destroy the undesirable contaminators in tuber-

culous materials exerts a detrimental influence upon the bacilli, it is to be noted that the 5 per cent concentration is reduced to 2.5 per cent when added to an equal volume of the specimen being tested, and on dilution with 10 c.c. of 0.9 per cent sodium chloride solution, the concentration of oxalic acid becomes about 0.5 per cent which suffers a reduction to less than 0.1 per cent in being added as a few drops of residue planted on the glycerol water crystal violet potato cylinder medium containing, besides the potato, 1.5 c.c. of 6 per cent glycerol water. The fact that the oxalic acid is innocuous to the growth of the tubercle bacilli when used in concentrations even to an equal volume of saturated solution is also borne out by the tests with sputums recorded in Table I.

TABLE II

TUBERCULOSTATIC ACTION OF OXALIC ACID ON VIRULENT HUMAN TUBERCLE BACILLI GROWN ON GLYCEROL CRYSTAL VIOLET POTATO CYLINDER MEDIUMS AFTER EIGHT WEEKS AT 37° C.

AMOUNT OF OXALIC ACID IN 1 C.C. 6 PER CENT GLYCEROL ADDED TO CRYSTAL VIOLET POTATO CYLINDER MEDIUM	AMOUNT OF SUSPENSION IN MG. OF TUBERCLE BACILLI PER C.C. USED FOR SEEDING CULTURE TUBES		
	1.0	0.000,1	0.000,001
Control Glycerol Crystal Violet Potato Cylinder Medium	4*	2	1
5 per cent Oxalic Acid	0	0	0
1 per cent Oxalic Acid	2	1	0
0.1 per cent Oxalic Acid	4	2	1
0.001 per cent Oxalic Acid	4	2	1
0.000,1 per cent Oxalic Acid	4	2	1

*The amount of growth obtained on the medium is arbitrarily graded from "0" =no appreciable macroscopic growth visible to "4" = a heavy and profuse growth over the entire medium.

In order to carry the comparison of tuberculostatic action of the oxalic acid and the sulphuric acid out more exactly another experiment was performed in which these acids in graded amounts were added to the 6 per cent glycerol water which was then used in the previously prescribed amounts (1 to 1½ c.c.) incorporated with the crystal violet potato cylinder in a 6 by ¾ inch bacteriologic culture tube. These culture tubes were then planted with graded amounts of suspensions of virulent human tubercle bacilli (Gluckson), incubated at 37° C. and read at weekly intervals. As control the 6 per cent glycerol water crystal violet potato cylinder medium was used. The results of this study are recorded in Table II-A giving the readings after eight weeks' incubation.

The findings recorded in Table II-A indicate that the sulphuric acid in like concentrations in the glycerol water added to the crystal violet potato cylinder mediums has a greater tuberculostatic action than the oxalic acid which is distinctly noticeable in the concentrations of 0.5 per cent and greater. Oxalic acid exerted no effect in 0.5 per cent concentration and both acids were without appreciable effect upon the growth of the virulent human tubercle bacilli in the concentration of 0.1 per cent or less.

Experiments are now in progress comparing oxalic and sulphuric acids for isolating tubercle bacilli from urine and animal tissues.

In tuberculostatic tests with virulent human tubercle bacilli as test organism and glycerol water crystal violet potato cylinder medium as nutrient, sodium oxalate proved far less toxic than oxalic and sulphuric acid in that even a one per cent concentration of this salt in the 6 per cent glycerol water exerted no retarding influence upon the growth of the bacilli while a 5 per cent concentration exerted only a slight retarding effect. However, sodium oxalate incorporated in 5 per cent glycerol broth agar was decidedly bacteriostatic in concentrations over one per cent but exerted no effect in 0.1 per cent concentration.

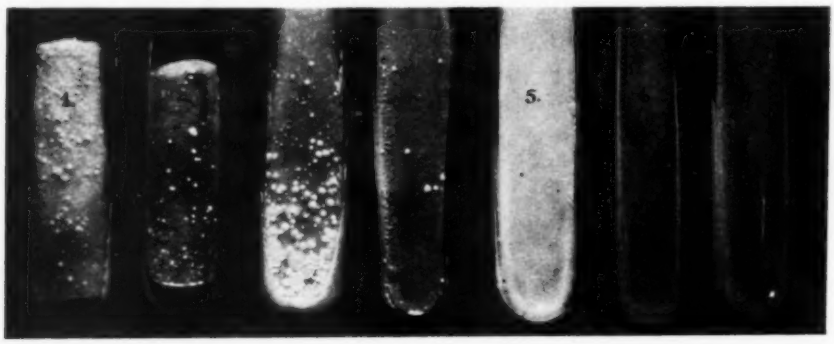


Fig. 3.—Growth of virulent avian tubercle bacilli on different mediums planted with a suspension containing 0.000,01 mg. per c.c. and incubated five weeks at 37° C. Note growth on all the potato mediums and on Petroff's medium, doubtful growth on Dorset's medium and no growth on Long's medium or glycerol agar. From left to right the tubes of medium are (1) glycerol water potato cylinder medium; (2) glycerol water crystal violet potato cylinder medium; (3) medium "P" (25 per cent ground potato, 2½ per cent glycerol, 1½ per cent agar; (4) Petroff's gentian violet egg medium; (5) Dorset's egg medium; (6) Long's nonprotein agar medium and (7) 5 per cent glycerol broth agar.

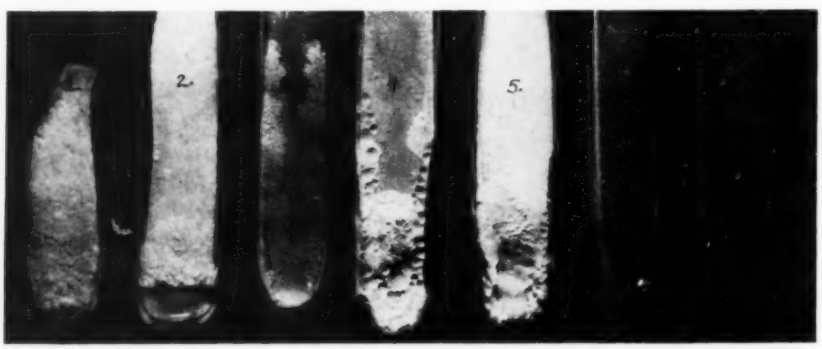


Fig. 4.—Growth of acid-fast grass bacilli on different mediums planted with a suspension containing 0.000,001 mg. per c.c. and incubated eight weeks at 37° C. Note growth on all the potato and egg mediums but none on the glycerol agar or Long's medium at this time. From left to right the tubes of medium are (1) glycerol water potato cylinder medium; (2) glycerol water crystal violet potato cylinder medium; (3) medium "P" (25 per cent ground potato, 2½ per cent glycerol, 1½ per cent agar); (4) Petroff's gentian violet egg medium; (5) Dorset's egg medium; (6) Long's nonprotein medium and (7) 5 per cent glycerol broth agar.

In view of the fact that in earlier studies with virulent human tubercle bacilli the effect of acetic acid as a reagent for destroying undesirable contaminators had not been recorded and considering the interest at this time of the effect of this acid upon human tubercle bacilli as compared to the effect

on other nonpathogenic and pathogenic acid-fast bacilli, the following experiment is now recorded. According to the usual procedure used by us for testing the effects of various concentrations of a reagent upon both the undesirable contaminators and the tubercle bacilli in a pathologic specimen, eight positive sputums were appropriately prepared and treated with an equal volume of varying concentrations of pure acetic acid from 2 to 20 per cent (by weight), and as control like specimens were treated with an equal volume of 6 per cent

TABLE II-A

TUBERCULOSTATIC ACTION OF OXALIC ACID IN GLYCEROL WATER CRYSTAL VIOLET POTATO MEDIUM COMPARED WITH THAT OF SULPHURIC ACID AFTER EIGHT WEEKS' INCUBATION AT 37° C.

PER CENT OXALIC OR SULPHURIC ACID IN 6 PER CENT GLYCEROL WATER ADDED TO MEDIUM	AMOUNT OF VIRULENT HUMAN TUBERCLE BACILLI (GLUCKSON) IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE TUBES		
	1.0	0.000,1	0.000,001
Control glycerol water crystal violet potato cylinder medium	4*	2	1
3.0 per cent Oxalic Acid	0	0	0
3.0 per cent Sulphuric Acid	0	0	0
1.0 per cent Oxalic Acid	2	1	0
1.0 per cent Sulphuric Acid	1	0	0
0.5 per cent Oxalic Acid	4	2	1
0.5 per cent Sulphuric Acid	3	1	0
0.1 per cent Oxalic Acid	4	2	1
0.1 per cent Sulphuric Acid	4	2	1

*The amount of growth obtained on the medium is graded from "0" = no appreciable macroscopic growth visible, to "4" = a heavy profuse growth. On concentration less than 0.1 per cent of added oxalic or sulphuric acid there occurred no retardation of growth.

TABLE II-B

THE EFFECT OF ACETIC ACID UPON THE MICROORGANISMS IN TUBERCULOUS SPUTUMS*

REAGENT USED FOR THE PRELIMINARY TREATMENT OF SPUTUMS BEFORE PLANTING ON GLYCEROL WATER CRYSTAL VIOLET POTATO CYLINDER MEDIUM	PURE CULTURES OF TUBERCLE BACILLI		UNDESIRABLE GROWTHS	
	NUMBER OF TUBES POSITIVE	PER CENT OF TOTAL TUBES POSITIVE†	NUMBER OF TUBES CONTAMINATED	PER CENT OF TUBES CONTAMINATED
Control 6 per cent Sulphuric Acid	30	75	10	25
20 per cent Acetic Acid	0	0	0	0
10 per cent Acetic Acid	3	8	5	12
7 per cent Acetic Acid	3	8	3	8
5 per cent Acetic Acid	2	5	7	18
2 per cent Acetic Acid	1	2	11	28

*Eight tuberculous sputums positive by microscopic examination were used in this study.

†A total of forty tubes of glycerol water crystal violet potato cylinder medium were used for the tests for each reagent and the percentages are figured on this basis.

sulphuric acid and incubated (thirty minutes at 37° C.), diluted (with 0.9 per cent saline solution), the centrifugated residues planted on five tubes of glycerol water crystal violet potato cylinder medium for each dilution of acid used and for each specimen of sputum being tested. The results of these tests are briefly recorded in Table II-B.

It is evident from the results of the study with acetic acid recorded in Table II-B that acetic acid even in concentrations as low as 2 per cent added in equal volume to tuberculous sputums and incubated for thirty minutes has a deleterious effect upon the human tubercle bacilli present in the sputums.

II. THE GROWTH OF ACID-FAST NONPATHOGENS ON VARIOUS NUTRIENT MEDIUMS AND THEIR REACTIONS TO VARIOUS CHEMICAL REAGENTS

Within the past few years Sewall and De Savitsch[4] reported on an interesting phenomenon of antagonism occurring between smegma bacilli and tubercle bacilli, and Dr. Sewall suggested the desirability of learning more about the reactions of the nonpathogenic acid-fast bacilli, and particularly the smegma bacillus, to various chemical reagents and especially so since the usual

Fig. 5.—Growth of acid-fast butter bacilli on different mediums planted with a suspension containing 0.000,001 mg. per c.c. and incubated four weeks at 37° C. Note growth on all the potato and egg mediums at this time and its absence on glycerol agar and Long's medium. From left to right the tubes of medium are (1) Long's nonprotein agar medium; (2) glycerol water potato cylinder medium; (3) glycerol water crystal violet potato cylinder medium; (4) medium "P" (25 per cent ground potato, 2½ per cent glycerol, 1½ per cent agar); (5) Petroff's gentian violet egg medium; (6) Dorset's egg medium, and (7) 5 per cent glycerol broth agar.

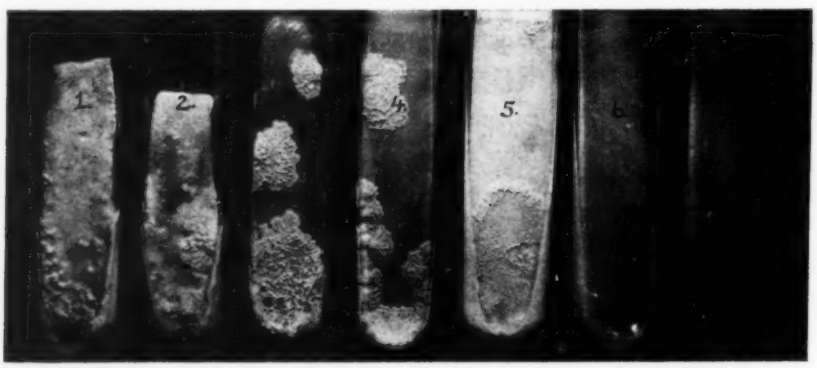


Fig. 6.—Growth of acid-fast "Day" bacilli on different mediums planted with a suspension containing 0.000.001 mg. per c.c. and incubated four weeks at 37° C. Note growth on all the potato and egg mediums at this time and none on the glycerol agar or Long's medium. From left to right the tubes of medium are (1) glycerol water potato cylinder medium; (2) glycerol water crystal violet potato cylinder medium; (3) medium "P" (25 per cent ground potato, 2½ per cent glycerol, 1½ per cent agar); (4) Petroff's gentian violet egg medium; (5) Dorset's egg medium; (6) Long's nonprotein agar medium; and (7) 5 per cent glycerol broth agar.

methods for isolating tubercle bacilli were not applicable to the isolation of smegma bacilli and since there existed no certain and reliable method for the primary isolation of this microorganism and investigations concerning the smegma bacilli usually had to be confined to the use of old laboratory strains of uncertain heritage. To learn more about the reactions to chemicals and the

nutrient requirements of the more common nonpathogenic acid-fast bacilli and especially as these may differ from the pathogenic tubercle bacilli was the purpose of the following studies.

A. Growth of Nonpathogenic Acid-Fast Bacilli on Various Mediums.—In order to determine the nutrient efficiency of the different mediums, graded suspensions of the various nonpathogenic acid-fast bacilli were carefully prepared, and with these suspensions, the different mediums were planted and were then incubated at 37° C. and the amount of growth recorded at weekly intervals. The mediums tested were those used in our earlier studies with

Fig. 7.—Growth of acid-fast timothy bacilli on different mediums planted with a suspension containing 0.000,001 mg. per c.c. and incubated four weeks at 37° C. Note growth on all the potato and egg mediums at this time and none on the glycerol agar or Long medium. From left to right the tubes of medium are (1) glycerol water potato cylinder medium; (2) glycerol water crystal violet potato cylinder medium; (3) medium "P" (25 per cent ground potato, 2½ per cent glycerol, 1½ per cent agar); (4) Petroff's gentian violet egg medium; (5) Dorset's egg medium; (6) Long's nonprotein agar medium; and (7) 5 per cent glycerol broth agar.

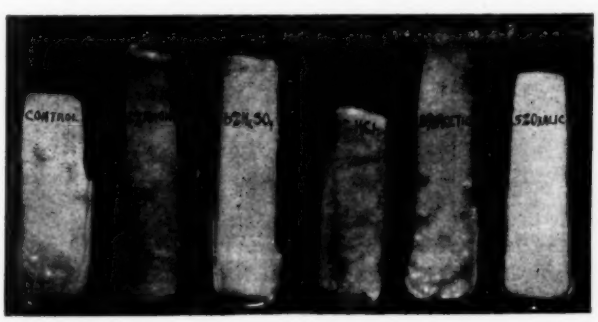


Fig. 8.—Reaction of smegma bacilli to various chemical reagents as determined by their growth after five weeks at 37° C. on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.000,1 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution, the sediment after centrifugation being planted. Note the growth on the control potato medium tube and on the tube which received the bacilli treated with the acetic acid reagent but not with the other reagents.

tubercle bacilli and included Long's nonprotein agar medium[5] containing 2½ per cent glycerol, 5 per cent glycerol broth agar medium, Dorset's egg medium,[6] Petroff's gentian violet egg medium,[7] a 6 per cent glycerol water plain potato cylinder medium, 6 per cent glycerol water crystal violet potato cylinder medium,[3] and a ground potato glycerol agar medium containing 25 per cent by weight of ground autoclaved potato, 2½ per cent glycerol and 1½ per cent agar (the latter labeled Medium "P" in Tables III to X).

The acid-fast bacilli used in this study included a smegma bacillus (Hygienic Laboratory strain), an avirulent avian bacillus (obtained from Dr. Esmond R. Long of the University of Chicago), a virulent avian bacillus (obtained from Dr. Wm. H. Feldman of the Mayo Foundation), a grass bacillus (from Dr. Long), two rapidly growing acid-fast bacilli "K" and "Day" (obtained from Dr. A. A. Day of Northwestern University), a butter bacillus (from Dr. Long) and a timothy bacillus (obtained from Dr. S. A. Petroff of Trudeau Sanatorium). All of these bacilli except the virulent avian strain from Dr. Feldman have been maintained in our laboratory for from at least two to five years. The graded seedings with suspensions of the different bacilli on the different mediums were made in gradations of one to ten dilutions usually from amounts of 1 mg. per c.c. to 0.000,000,001 mg. per c.c., each complete experiment with one strain of bacilli being run in duplicate and each dilution of seeding being tested by using four tubes of medium thus making duplicate experiments with quadruplicate tubes. The tabulations are recorded for simplicity as "+," indicating a definite positive growth of the bacilli on all the tubes planted, "?" indicating that not all the tubes were regularly positive or that one experiment proved positive and the duplicate negative, while "0" indicated that growth was absent from all the tubes planted in both experiments. The culture tubes were read at weekly intervals for a period of twelve weeks at incubator temperature (37° C.). In these experiments no particular significance to the speed of growth was noted except in so far that most of the culture tubes planted with the heavier suspensions in the particular case under consideration revealed growth within two to three weeks with these rapid growers while with the relatively smaller seedings growth occurred even only after four weeks incubation. In Table III is recorded the findings obtained with the rapidly growing acid-fast smegma bacillus (Hygienic Laboratory strain).

TABLE III

GROWTH OF SMEGMA BACILLI (HYGIENIC LABORATORY) ON DIFFERENT MEDIUMS PLANTED WITH SUSPENSIONS CONTAINING GRADED AMOUNTS OF BACILLI

MEDIUM USED FOR GROWTH	AMOUNT OF BACILLI IN MG. IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES					
	0.01	0.001	0.000,1	0.000,01	0.000,001	0.000,000,1
Glycerol water potato cylinder medium	+*	+	?	?	0	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0	0
Medium "P" (25 per cent ground potato, 2.5 per cent glycerol and 1.5 per cent agar)	+	+	+	+	0	0
Dorset's egg medium	+	+	+	+	0	0
Petroff's gentian violet egg medium	+	+	+	+	0	0
Long's nonprotein agar medium	+	?	?	0	0	0
5 per cent glycerol broth agar medium	+	+	+	?	0	0

*The results recorded in growth of bacilli are given as the average of two experiments, four tubes being seeded for each medium and each dilution of bacilli used in each experiment; + indicating all the tubes revealed growth within twelve weeks' incubation; ? indicating that some of the tubes or one experiment proved negative and 0 that all the tubes were negative.

The smegma bacilli (Hygienic Laboratory strain) tested (results recorded in Table III) grew well and with seedings as low as 0.000,01 mg. per c.c. on Medium "P" (consisting of autoclaved ground potato, glycerol and agar), Dorset's egg medium and Petroff's gentian violet egg medium while on glycerol agar and the potato cylinder mediums certain growth occurred only with heavier seedings (0.001 mg. or heavier suspensions) and as a nutrient Long's medium proved least suitable requiring seedings with suspensions of smegma bacilli heavier than 0.001 mg. per c.c.

Fig. 9.—Reaction of avirulent acid-fast avian bacilli to various chemical reagents as determined by their growth after nine weeks at 37° C. on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.01 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution, the sediment after centrifugation being planted. Note the growth on the control potato medium tube and on the tubes which received the bacilli treated with the acetic and sulphuric acid reagents but not with the sodium hydroxide reagent. Questionable results occur with the suspension of these bacilli when the oxalic acid reagent is used.

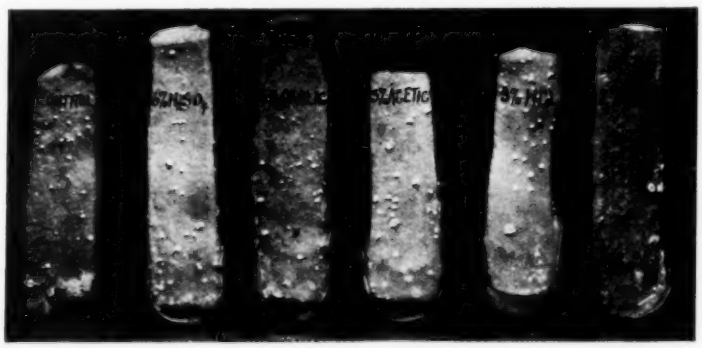


Fig. 10.—Reaction of virulent avian tubercle bacilli to various chemical reagents as determined by their growth after five weeks at 37° C. on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.000,1 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution, the sediment after centrifugation being planted. Note the peculiar characteristic of this bacillus in being unaffected by practically all the reagents and growing in all the tubes.

The avirulent avian acid-fast bacilli grew on these mediums with smaller seedings than did the smegma bacilli, as is to be noted from the data recorded in Table IV.

The glycerol water potato cylinder medium and Medium "P" proved best suited for supporting the growth of the avirulent avian tubercle bacilli, a seeding with suspensions containing 0.000,000,1 mg. per c.c. still give posi-

tive growths on this medium. Long's medium proved least suitable for the growth of these bacilli (growing only when seeded with suspensions containing 0.000,01 mg. per c.c.) while the glycerol water crystal violet potato cylinder medium, Dorset's medium, Petroff's medium and glycerol agar occupied an intermediate position.

Contrasting with all the strains of rapidly growing acid-fast bacilli reported on in this study the virulent avian tubercle bacilli isolated by Dr.

TABLE IV

THE GROWTH OF AVIRULENT AVIAN ACID-FAST BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR GROWTH	AMOUNT OF BACILLI IN MG. IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES				
	0.000,1	0.000,01	0.000,001	0.000,000,1	0.000,000,01
Glycerol water potato cylinder medium	+*	+	+	+	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium "P" (25 per cent potato, 2.5 per cent glycerol and 1.5 per cent agar)	+	+	+	+	0
Dorset's egg medium	+	+	+	?	0
Petroff's gentian violet egg medium	+	+	+	?	0
Long's nonprotein agar medium	+	+	0	0	0
5 per cent glycerol broth agar medium	+	+	+	?	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

TABLE V

THE GROWTH OF VIRULENT AVIAN TUBERCLE BACILLI (FELDMAN) ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG. IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES								
	1.0	0.1	0.01	0.001	0.000,1	0.000,01	0.000,001	0.000,000,1	0.000,000,01
Glycerol water potato cylinder medium	+	+	+	+	+	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	+	+	+	+	?	0
Medium "P" (25 per cent potato, 2.5 per cent glycerol and 1.5 per cent agar)	+	+	+	+	+	+	+	?	0
Dorset's egg medium	+	+	+	+	+	?	?	0	0
Petroff's gentian violet egg medium	+	+	+	+	+	+	?	0	0
Long's nonprotein agar medium	+	?	?	0	0	0	0	0	0
5 per cent glycerol broth agar medium	+	+	?	?	0	0	0	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

Wm. H. Feldman,* and proved highly pathogenic for chickens in recent tests by Dr. Henry Sewall, revealed a striking favorable growth-promoting action of all the potato mediums for these bacilli as is noted from the findings recorded in Table V.

It is to be noted from the data recorded in Table V that the virulent avian tubercle bacilli (Feldman) will grow on all the potato mediums when planted with suspensions containing as little as 0.000,000,1 mg. of bacilli

TABLE VI

THE GROWTH OF ACID-FAST GRASS BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG. IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES				
	0.000,1	0.000,01	0.000,001	0.000,000,1	0.000,000,01
Glycerol water potato cylinder medium	+*	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium "P" (25 per cent potato, 2.5 per cent glycerol and 1.5 per cent agar)	+	+	+	?	0
Dorset's egg medium	+	+	+	?	0
Petroff's gentian violet egg medium	+	+	+	?	0
Long's nonprotein agar medium	+	?	?	0	0
5 per cent glycerol broth agar medium	+	?	?	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

TABLE VII

THE GROWTH OF ACID-FAST NONPATHOGENIC "K" BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG. IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES				
	0.000,1	0.000,01	0.000,001	0.000,000,1	0.000,000,01
Glycerol water potato cylinder medium	+*	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium "P" (25 per cent potato, 2.5 per cent glycerol and 1.5 per cent agar)	+	+	+	?	0
Dorset's egg medium	+	+	?	0	0
Petroff's gentian violet egg medium	+	+	?	0	0
Long's nonprotein agar medium	+	?	?	0	0
5 per cent glycerol broth agar medium	+	+	?	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

while the egg mediums require a seeding with 0.000,001 mg. suspensions and glycerol agar and Long's medium about 0.01 mg. suspensions. These findings closely parallel those with virulent human and bovine tubercle bacilli previ-

*Note Dr. Wm. H. Feldman isolated this strain and a large number of other avian strains from pathologic material by means of the new sulphuric acid crystal violet potato cylinder method for isolating tubercle bacilli recently described by us.

ously recorded by us[8] and explains the success achieved by Dr. Feldman in isolating avian tubercle bacilli from pathologic materials by the use of the sulphuric acid crystal violet potato cylinder method.

Similar experiments concerned with the growth of the grass bacilli, the "K" bacilli, the butter bacilli and the Day bacilli on the various mediums are recorded in Tables VI to IX.

The acid-fast rapidly growing grass bacilli (see Table VI) grows with equal facility on the potato and egg mediums tested in that growth was still obtained with suspensions containing as low as 0.000,000,1 mg. bacilli per c.c. while on Long's nonprotein medium and glycerol agar growth occurred only with slightly heavier seedings (about 0.000,001 mg. per c.c. suspensions).

TABLE VIII

THE GROWTH OF ACID-FAST "BUTTER" BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG. IN SUSPENSION USED FOR SEEDING CULTURE TUBES			
	0.000,01	0.000,001	0.000,000,1	0.000,000,01
Glycerol water potato cylinder medium	+*	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	?	0
Medium "P" (25 per cent potato, 2.5 per cent glycerol and 1.5 per cent agar)	+	+	?	0
Dorset's egg medium	+	+	?	0
Petroff's gentian violet egg medium	+	+	?	0
Long's nonprotein agar medium	+	?	0	0
5 per cent glycerol broth agar medium	+	?	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

The acid-fast nonpathogenic "K" bacillus (see Table VII) grew with equal facility on all the potato mediums (with suspensions of 0.000,000,1 mg.), less so on the egg mediums (with suspensions of 0.000,001 mg.) and about equally so on Long's medium and 5 per cent glycerol broth agar. Although

TABLE IX

THE GROWTH OF ACID-FAST NONPATHOGENIC "DAY" BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG. IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES				
	0.000,1	0.000,01	0.000,001	0.000,000,1	0.000,000,01
Glycerol water potato cylinder medium	+*	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium "P" (25 per cent potato, 2.5 per cent glycerol and 1.5 per cent agar)	+	+	+	?	0
Dorset's egg medium	+	+	+	?	0
Petroff's gentian violet egg medium	+	+	+	?	0
Long's nonprotein agar medium	+	+	?	0	0
5 per cent glycerol broth agar medium	+	?	0	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

there is a certain parallelism with the growth of the grass bacillus, the egg mediums proved slightly less suitable for this organism ("K") than the potato mediums and the glycerol broth agar and Long's nonprotein medium about equal to the egg mediums.

The growth of the acid-fast butter bacillus tested on the potato and egg mediums proved to be about the same in that suspensions containing 0.000,000,1 mg. per c.c. still were able to grow on these mediums. The ability of these bacilli to draw upon a wide and limited nutrient source is indicated by their ability to grow on Long's medium and glycerol broth agar in almost as low seedings as upon the potato and egg mediums which is in striking contrast to the slow-growing pathogenic avian, bovine and human tubercle bacilli. There is also a striking parallelism between the growth of the butter bacillus and the grass bacillus (Table VI).

The growth of the acid-fast nonpathogenic Day bacilli resembled that of the rapidly growing acid-fast grass and butter bacilli on the potato and egg mediums in that growth occurred with the 0.000,000,1 mg. suspensions; Long's medium proved a little less suitable and with this organism glycerol broth agar proved least suitable.

TABLE X

THE GROWTH OF ACID-FAST TIMOTHY BACILLI ON DIFFERENT MEDIUMS PLANTED WITH GRADED SUSPENSIONS

MEDIUM USED FOR CULTURE	AMOUNT OF BACILLI IN MG. IN SUSPENSION USED FOR SEEDING THE CULTURE TUBES				
	0.000,1	0.000,01	0.000,001	0.000,000,1	0.000,000,01
Glycerol water potato cylinder medium	+*	+	+	?	0
Glycerol water crystal violet potato cylinder medium	+	+	+	?	0
Medium "P" (25 per cent potato, 2.5 per cent glycerol and 1.5 per cent agar)	+	+	+	?	0
Dorset's egg medium	+	+	+	?	0
Petroff's gentian violet egg medium	+	+	+	?	0
Long's nonprotein agar medium	+	?	0	0	0
5 per cent glycerol broth agar medium	+	+	?	0	0

*The markings are identical to those used in Table III and the description is given in the footnote of Table III.

The acid-fast timothy bacilli grew with equal facility on all the potato and egg mediums in suspensions as dilute as 0.000,000,1 mg. per c.c. thus resembling the other rapidly growing nonpathogenic acid-fast bacilli studied. Long's medium proved less serviceable than glycerol broth agar for supporting the growth of the timothy bacillus.

In summarizing the part of this study concerned with the growth of the rapidly growing acid-fast nonpathogenic bacilli, it is to be noted that practically all of these rapidly growing acid-fast nonpathogens studied grew well on either potato or egg mediums, in some cases slightly better on the potato mediums than on the egg mediums but in no case was a better growth noted on the egg mediums than on the potato mediums. The limit of growth on the

potato and egg mediums was with seedings of suspensions containing about 0.000,000,1 mg. of bacilli (a few drops of such suspensions being used for seeding). In the case of the smegma bacilli (Hygienic Laboratory Strain) heavier seedings were required even on the most suitable medium, a suspension containing 0.000,01 mg. per c.c. being required. The strain of virulent avian tubercle bacilli grew decidedly better on the potato mediums than on the other mediums including egg mediums in this respect resembling the virulent human and bovine strains of tubercle bacilli. A wide range of variability in growth of the nonpathogenic acid-fast bacilli on Long's nonprotein medium and glycerol broth agar was found but in no case was the contrast

Fig. 11.—Reaction of **acid-fast grass bacilli to various chemical** reagents as determined by their growth after three **weeks at 37° C. on glycerol water crystal** violet potato cylinder medium following treatment of a suspension containing 0.000,1 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution, the sediment after centrifugation being planted. Note the growth of the control potato medium tube and on the tube planted with the bacilli treated with the acetic acid reagent but not with the sulphuric acid or sodium hydroxide reagents.

Fig. 12.—**Reaction of acid-fast "K" bacilli to various chemical reagents as determined** by their growth after seven weeks at 37° C. on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.01 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution, the sediment after centrifugation being planted. Note the growth with this suspension at this time on the control tube and with those treated with the acetic, sulphuric and oxalic reagents but not with the hydrochloric acid and sodium hydroxide reagents.

with the potato and egg mediums as striking as with the pathogenic human, bovine and avian bacilli in which case relatively heavy seedings were required to obtain growth on Long's medium and glycerol agar.

B. Relative Resistance of Nonpathogenic Acid-Fast Bacilli to the Action of Certain Chemical Reagents.—In order to extend the observations previously recorded[1, 3] on the effect of various chemical reagents on the pathogenic human and bovine tubercle bacilli the following experiments were performed

with the rapidly growing nonpathogenic acid-fast bacilli. In addition they were also extended to include the effect on a virulent strain of avian tubercle bacilli for comparison. The reagents used included 6 per cent sulphuric acid, 5 per cent oxalic acid, 3 per cent acetic acid, 2 per cent sodium hydroxide, and 3 per cent hydrochloric acid. The reagents were added in equal volume (1 c.c.) to fine suspensions of the bacilli in sterile 0.9 per cent sodium chloride solution containing from 1.0 to 0.000,001 mg. per c.c. The mixture of reagent and bacillary suspension was shaken and placed in an incubator for thirty

Fig. 13.—Reaction of acid-fast butter bacilli to various chemical reagents as determined by their growth after four weeks at 37° C. on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.01 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution, the sediment after centrifugation being planted. Note especially the striking contrast between the lack of toxicity of the acetic acid reagent to these bacilli as compared to other reagents.

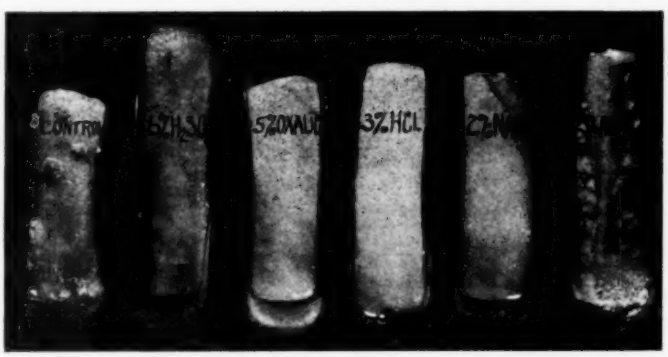


Fig. 14.—Reaction of acid-fast timothy bacilli to various chemical reagents as determined by their growth after four weeks at 37° C. on glycerol water crystal violet potato cylinder medium following treatment of a suspension containing 0.000.1 mg. per c.c. with an equal volume of the reagent (designated in the photograph) for thirty minutes at 37° C. and diluting with sterile saline solution, the sediment after centrifugation being planted. Note especially the striking contrast between the lack of toxicity of the acetic acid reagent to these bacilli as compared to the other reagents.

minutes at 37° C., after which it was diluted with sterile saline solution, centrifugated and the sediment planted on glycerol water crystal violet potato cylinder medium. Weekly records of growth were made. The concentration of the reagents used was determined by previous experiences in the study of the effect of these reagents upon human and bovine tubercle bacilli, the concentrations being chosen which were innocuous to tubercle bacilli and

which were still capable of destroying the majority of the contaminating microorganisms usually present in tuberculous sputums. An exception, however, was included in using 3 per cent acetic acid which in an earlier part of this paper was shown to have a deleterious influence upon human tubercle bacilli. It might be noted here also that the bactericidal action of all these reagents toward the contaminating microorganisms present in tuberculous sputums was practically the same for all the reagents when used in the concentrations (by weight) specified in the tabulations.

TABLE XI

REACTION OF SMEGMA BACILLI (HYGIENIC LABORATORY STRAIN) TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG. PER C.C. OF SUSPENSION USED FOR TESTING			
	1.0	0.01	0.000,1	0.000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	2	?†	0	0
5 per cent Oxalic Acid	2	?	0	0
3 per cent Acetic Acid	3	1	?	?
3 per cent Hydrochloric Acid	0	0	0	0
2 per cent Sodium Hydroxide	0	0	0	0

*The readings in growth on the glycerol water crystal violet potato cylinder medium is graded from "0" = no appreciable macroscopic growth to "4" = a good profuse growth, and the readings are an average of four tubes of glycerol water crystal violet potato cylinder medium planted and in duplicate experiment observed for ten weeks at 37° C.

† "?" indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period.

TABLE XII

REACTION OF AVIRULENT ACID-FAST AVIAN BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG. PER C.C. OF SUSPENSION USED FOR TESTING			
	1.0	0.01	0.000,1	0.000,001
Control	4*	3	1	?†
6 per cent Sulphuric Acid	2	1	0	0
5 per cent Oxalic Acid	2	?	0	0
3 per cent Acetic Acid	3	2	?	0
3 per cent Hydrochloric Acid	?	0	0	0
2 per cent Sodium Hydroxide	?	0	0	0

*The readings are graded from "0" = no macroscopic growth to "4" = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium.

†"?" indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period.

The same nonpathogenic acid-fast bacilli that were included in the study with the culture mediums reported upon earlier in this paper were tested in the following studies: they included the smegma bacillus (Hygienic Laboratory Strain), the avirulent acid-fast avian bacillus, the virulent avian tubercle bacillus (Feldman), the acid-fast grass bacillus, the "K" bacillus, the acid-fast butter bacillus, the acid-fast Day bacillus, and the acid-fast timothy bacillus. The results of these studies are recorded in Tables XI to XVIII.

It is to be noted from the study recorded in Table XI that the smegma bacillus is most resistant to the 3 per cent acetic acid while it is least resistant to 2 per cent sodium hydroxide and 3 per cent hydrochloric acid, with the 6 per cent sulphuric and 5 per cent oxalic acids lying in an intermediate position. The reaction of the smegma bacilli to the acetic acid is especially

interesting since this acid proved relatively highly toxic to human tubercle bacilli, and if the smegma bacillus (Hygienic Laboratory Strain) is representative of the various strains existing, the possibility suggests itself that this reagent may prove serviceable for the isolation of this microorganism from human sources.

There is to be noted a striking parallelism between the reaction of the avirulent avian bacilli (Table XII) and the smegma bacilli (Table XI) both in reaction to the chemical reagents tested and in growth behavior (Tables III and IV) on the various mediums.

TABLE XIII

REACTION OF VIRULENT AVIAN TUBERCLE BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG. PER C.C. OF SUSPENSION USED FOR TESTING			
	1.0	0.01	0.000,1	0.000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	4	3	2	1
5 per cent Oxalic Acid	4	3	2	1
3 per cent Acetic Acid	4	3	2	1
3 per cent Hydrochloric Acid	4	3	2	?†
2 per cent Sodium Hydroxide	4	3	2	?

*The readings are graded from "0" = no macroscopic growth to "4" = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium.

†"?" indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period.

TABLE XIV

REACTION OF GRASS BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG. PER C.C. OF SUSPENSION USED FOR TESTING			
	1.0	0.01	0.000,1	0.000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	2	?†	0	0
5 per cent Oxalic Acid	1	?	0	0
3 per cent Acetic Acid	4	3	2	1
3 per cent Hydrochloric Acid	1	0	0	0
2 per cent Sodium Hydroxide	1	0	0	0

*The readings are graded from "0" = no macroscopic growth to "4" = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium.

†"?" indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period.

In contrast to the reaction of the avirulent acid-fast avian bacillus (Table XII) to the chemical reagents tested the virulent strain of avian tubercle bacilli reveals a decided resistance to the action to all the reagents indicating that any one of these reagents may be used for isolating these bacilli although the preference would seem to be for the sulphuric, oxalic or acetic acids as compared to the hydrochloric acid or sodium hydroxide. It is noteworthy also that the 3 per cent acetic acid proved innocuous in contrast to its action on human tubercle bacilli and in agreement with the effect upon the avirulent acid-fast avian bacilli and the smegma bacilli. This fact, if borne out with other avian strains, suggests a means of differentiating avian from human and bovine tubercle bacilli. This is now being further studied by us and will be reported on more fully at a later date.

The strain of grass bacilli tested (Table XIV) revealed a high resistance to the 3 per cent acetic acid reagent and a decided bactericidal effect of all the other reagents tested, thus showing a certain parallelism to the reaction of the Smegma bacilli (Table XI) and the avirulent acid-fast avian bacilli (Table XII).

TABLE XV

REACTION OF ACID-FAST "K" BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG. PER C.C. OF SUSPENSION USED FOR TESTING			
	1.0	0.01	0.000,1	0.000,001
Control	4*	3	2	?†
6 per cent Sulphuric Acid	3	1	0	0
5 per cent Oxalic Acid	2	?	0	0
3 per cent Acetic Acid	3	2	0	0
3 per cent Hydrochloric Acid	?	0	0	0
2 per cent Sodium Hydroxide	0	0	0	0

*The readings are graded from "0" = no macroscopic growth to "4" = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium.

†"?" indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period.

TABLE XVI

REACTION OF ACID-FAST BUTTER BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG. PER C.C. OF SUSPENSION USED FOR TESTING			
	1.0	0.01	0.000,1	0.000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	2	1	?†	0
5 per cent Oxalic Acid	1	?	0	0
3 per cent Acetic Acid	3	2	2	?
3 per cent Hydrochloric Acid	1	0	0	0
2 per cent Sodium Hydroxide	1	0	0	0

*The readings are graded from "0" = no macroscopic growth to "4" = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium.

†"?" indicates that in the dilution specified only part of the tubes revealed growth within ten weeks incubation period.

TABLE XVII

REACTION OF ACID-FAST "DAY" BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG. PER C.C. OF SUSPENSION USED FOR TESTING			
	1.0	0.01	0.000,1	0.000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	3	2	1	0
5 per cent Oxalic Acid	3	2	1	0
3 per cent Acetic Acid	4	3	2	1
3 per cent Hydrochloric Acid	0	0	0	0
2 per cent Sodium Hydroxide	0	0	0	0

*The readings are graded from "0" = no macroscopic growth to "4" = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium.

The acid-fast "K" bacillus revealed a striking susceptibility to all the reagents tested, it being more pronounced with hydrochloric acid and sodium hydroxide. Even the 3 per cent acetic acid reagent which displayed a lack of toxicity to the majority of the acid-fast nonpathogenic bacilli also displayed a distinct toxicity to the "K" bacillus.

It is to be noted from the results recorded in Table XVI that there is a striking resemblance between the reaction of the acid-fast butter bacilli to that of the acid-fast grass bacilli (Table XIV).

The reaction of the acid-fast Day bacilli would appear different from some of the other rapid growers tested in that in addition to slight toxicity of the sulphuric acid and oxalic acid reagents, the acetic acid reagent proved entirely innocuous, while both the hydrochloric acid and sodium hydroxide reagents displayed a profound effect upon these bacilli, thus differing from the rapidly growing "K" and butter bacilli.

TABLE XVIII

THE REACTION OF ACID-FAST TIMOTHY BACILLI TO VARIOUS CHEMICAL REAGENTS

REAGENT USED IN EQUAL VOLUME ADDED TO SUSPENSION OF BACILLI	AMOUNT OF BACILLI IN MG. PER C.C. OF SUSPENSION USED FOR TESTING			
	1.0	0.01	0.000,1	0.000,001
Control	4*	3	2	1
6 per cent Sulphuric Acid	2	1	0	0
5 per cent Oxalic Acid	2	1	0	0
3 per cent Acetic Acid	4	3	2	1
3 per cent Hydrochloric Acid	0	0	0	0
2 per cent Sodium Hydroxide	1	0	0	0

*The readings are graded from "0" = no macroscopic growth to "4" = a profuse heavy growth occurring on the glycerol water crystal violet potato cylinder medium.

There is a distinct resemblance of the reaction of the acid-fast timothy bacillus (Table XVIII) to the various reagents tested and that of the smegma bacillus (Table XI), the acid-fast grass bacillus (Table XIV), the acid-fast butter bacilli (Table XVI), and the Day bacilli (Table XVII) in that the acetic acid reagent is more innocuous to these bacilli than the sulphuric acid or oxalic acid reagents, while the hydrochloric acid and the sodium hydroxide reagents proved decidedly toxic to all these strains.

In summarizing the results of these studies on the reactions to various chemical reagents of the rapidly growing acid-fast bacilli as compared to the virulent avian tubercle bacilli and the pathogenic human and bovine tubercle bacilli it is to be noted particularly that there is a striking resemblance in the reaction of all the nonpathogens in that they display a decided resistance to the action of the 3 per cent acetic acid reagent and a pronounced susceptibility to the 3 per cent hydrochloric acid reagent, and the 2 per cent sodium hydroxide reagent. The reaction of these bacilli to the 6 per cent sulphuric acid reagent and the 5 per cent oxalic acid reagent lies intermediate to the effect of the acetic acid and the hydrochloric acid or sodium hydroxide reagents. In the case of the grass, butter, Day and timothy bacilli, the 3 per cent acetic acid proved so innocuous that these bacilli even in high dilutions grew about as well as the untreated controls on the glycerol water crystal violet potato cylinder medium. It was interesting to note that the virulent strain of avian tubercle bacilli tested proved highly resistant to the action of all the chemical reagents tested which suggested the possibility of utilizing this fact for differentiating these bacilli culturally from the human and bovine tubercle bacilli especially if this fact is borne out in further tests with other virulent strains of avian tubercle bacilli. The reaction of the rapidly growing

acid-fast bacilli to the sulphuric and oxalic acid reagents also offered an explanation for the fact that only the pathogens in contaminated tuberculous materials would survive this treatment, and thus account for the reliability of the sulphuric or oxalic acid crystal violet potato cylinder method for diagnostic purposes as well as elucidating the fact that this procedure proved impractical for the isolation of smegma bacilli in our hands. On the basis of these observations it seems likely that the 3 per cent acetic acid reagent may prove serviceable for isolating smegma bacilli from human sources and work is now in progress to test this out.*

SUMMARY AND CONCLUSIONS

1. In comparative tests a 5 per cent oxalic acid reagent proved superior to a 6 per cent sulphuric acid reagent for isolating tubercle bacilli from tuberculous sputums in that a greater percentage of the total tubes planted yielded positive cultures of tubercle bacilli which was accounted for by the lesser toxicity of the oxalic reagent as determined by bacteriostatic tests and also due to a greater germicidal action of this reagent on contaminating organisms. The oxalic acid reagent also possessed advantages over the sulphuric acid as a reagent inherent in being a solid easily weighed, stable and obtainable in pure crystalline form.

2. A study of the quantitative growth characteristics of a group of nonpathogenic acid-fast bacilli including a strain of smegma, grass, butter, timothy, "K," "Day" and an avirulent avian bacilli on various nutrient mediums used for growing tubercle bacilli revealed that these bacilli grow better on potato and egg mediums as compared to glycerol broth agar and a nonprotein agar medium (Long's) especially when the culture tubes are planted with small numbers of bacilli. The same proved true of a virulent avian tubercle bacillus studied. Potato or egg mediums are therefore suggested as most suited to the isolation of these bacilli when present in small numbers. With heavy seedings they grew about equally well on all the mediums tested.

3. In a study of the reaction of the nonpathogenic acid-fast bacilli to various chemical reagents, it was found that a 3 per cent acetic acid reagent added in equal volume to different amounts of fine suspensions of these bacilli proved comparatively innocuous while capable of efficiently destroying the usual contaminating microorganisms in tuberculous sputums, suggesting that this acid should prove serviceable for the isolation of this class of microorganisms and particularly the smegma bacillus.

The lack of toxicity of the 3 per cent acetic acid reagent to the rapidly growing acid-fast bacilli and a strain of virulent avian tubercle bacilli is in striking contrast to its toxic effect upon virulent human and bovine tubercle bacilli, and if borne out with more extensive tests with strains of virulent avian tubercle bacilli suggests the use of this reagent for differentiating this microorganism culturally from the other two pathogenic varieties.

A 3 per cent hydrochloric acid reagent and a 2 per cent sodium hydroxide reagent proved highly toxic to all the nonpathogenic acid-fast bacilli tested,

*The authors are grateful to Mr. L. D. Miller for his kind assistance in pursuing the technical details of this study.

while a 6 per cent sulphuric acid reagent and a 5 per cent oxalic acid reagent usually occupied an intermediate position.

In contrast to both the pathogenic human and bovine tubercle bacilli which were highly susceptible to the action of the acetic acid reagent and resistant to the sulphuric and oxalic acid reagents and the nonpathogenic acid-fast bacilli that displayed a decided resistance to the acetic acid reagent and were sensitive to the sulphuric acid and oxalic acid reagents, the virulent strain of avian tubercle bacilli tested proved resistant to all the acid reagents tested as well as the 2 per cent sodium hydroxide reagent.

REFERENCES

1. Corper, H. J., and Uyei, Nao: The Cultivation of Tubercle Bacilli; an Improved Method for Isolation from Tuberculous Materials, J. LAB. & CLIN. MED. **13**: 469-480, 1928, and Further Observations With a New Method for Cultivating Tubercle Bacilli: A Comparison With Guinea Pig Inoculation and Petroff's Method, Ibid. **14**: 393, 1929.
2. Corper, H. J.: The Certified Diagnosis of Tuberculosis, Practical Evaluation of a New Method for Cultivating Tubercle Bacilli for Diagnostic Purposes, J. A. M. A. **91**: 371, 1928.
3. Corper, H. J., and Uyei, Nao: A Simple Glycerol Water Crystal Violet Potato Cylinder Medium for Diagnostic Cultures of Tubercle Bacilli, Arch. Pathol. and Lab. Med. **7**: 835, 1929.
4. Sewall, Henry, and De Savitsch, Eugene: On the Biological Antagonism Between Smegma Bacillus and Tubercle Bacillus, National Tuberculosis Association, Transactions of the Twenty-Fourth Annual Meeting **24**: 234, 1928.
5. Long, Esmond R., and Seibert, Florence B.: The Chemical Nature of the Active Principle of Tuberculin, Tubercle **8**: 111, 1926.
6. Dorset, M.: The Use of Eggs as a Medium for the Cultivation of the Bacillus Tuberculosis, Am. Med. (Philadelphia) **3**: 555, 1902.
7. Petroff, S. A.: A New and Rapid Method for the Isolation and Cultivation of Tubercle Bacilli Directly from the Sputum and Feces, J. Exper. Med. **21**: 38, 1915; Some Cultural Studies on Tubercle Bacilli, Bull. Johns Hopkins Hosp. **26**: 276, 1915.
8. Corper, H. J., and Uyei, Nao: The Isolation of Tubercle Bacilli from Contaminated Tuberculous Materials, Am. Rev. Tuberculosis **16**: 299, 1927.

IMPROVED COLORIMETRIC PROCEDURES FOR THE QUANTITATIVE ESTIMATION OF THE PROTEINS OF THE CEREBROSPINAL FLUID*

By Philip B. Matz, M.D., Washington, D. C., and
Nathan Novick, Clinical Laboratorian, Hines, Illinois

ORIGIN OF PROTEINS

Fremont-Smith and Ayer[1] maintain that the origin of the proteins normally present in the cerebrospinal fluid is largely a matter of conjecture. The ventricular fluid contains much less protein than the lumbar fluid. These observers state that it is possible that the protein of normal ventricular fluid comes through the chorioid plexus from the blood plasma, or that it is a product of the ependymal cells. It probably represents the minimal portion of the perivascular fluid which enters the ventricles. The amount of proteins in the normal ventricular fluid is very slight.

Fremont-Smith and Ayer hold that the increasing amount of protein found in the lumbar fluid may be due to the larger number of perivascular spaces emptying into the subarachnoid space. Other possible sources of the proteins may be the arachnoid cells, the lymph spaces within the nerve roots, and the central canal of the cord.

These observers maintain that, in view of the uncertainty as to the origin of the proteins normally found in the cerebrospinal fluid, it is impossible to come to a definite conclusion in regard to the source or sources of the proteins found in pathologic states. All of the possible sources mentioned for normal proteins and in addition transudation through dilated meningeal vessels may be the sources of the proteins in disease.

The relative proportions of albumin and globulin as found in the blood and the relative increase of globulin over albumin in the blood in disease would support the theory that a certain portion of the protein of the cerebrospinal fluid has its origin in the blood.

Ayer and Foster[2] hold that protein excess of the cerebrospinal fluid is an index not only of an exudative meningeal process, but also of the degree of permeability of meningeal vessels under pathologic or abnormal physiologic states. In the one case the protein is of exudative, in the other of transudative origin. The sources of origin are therefore diverse.

Hewitt[3] is of the opinion that a mere leakage phenomenon does not altogether account for the presence of the increased amount of proteins, since albumin with its smaller molecular size would probably diffuse more readily

*Read by title before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.

From the Research Subdivision Medical Service, U. S. Veterans Bureau and the Clinical Laboratory, Edward Hines, Jr. Hospital, Hines, Illinois.

Published with the permission of the Medical Director of the U. S. Veterans Bureau.

and thus the albumin-globulin ratio would increase and not decrease, as occurs in certain forms of neurosyphilis.

Robertson[4] holds that normally the globulin of the blood serum is less abundant than the albumin fraction. In animals or human beings infected with the streptococcus or staphylococcus, however, the ratio may be much more than unity.

The question of the cause of this change in the blood serum is of importance to our understanding of the mechanisms by which the organism protects itself against infections. In diphtheria, for instance, the antitoxins are found in the globulin fraction of the serum.

The cause of the increase of the globulin of the serum in infections is still unknown, although a number of observers have presented experimental theories which appear reasonable.

Buck, quoted by Robertson,[4] has shown that if ether or chloroform be administered for very prolonged periods to animals, so that albuminuria begins to appear, the globulin-quotient rises far more markedly than could be accounted for by an escape of serum albumin into the urine. This indicates that possibly the true source of the marked alterations in the globulin-quotient, which occur in infections, is the result of an increased permeability of the tissue cells.

It appears highly probable that one of the principal sources of the globulin of cerebrospinal fluid is the blood. If this is so, then the factors which are responsible for an increase of the globulin of the blood are also responsible for an increase of the globulin of the cerebrospinal fluid.

NATURE OF PROTEINS

Hewitt[3] found that the proteins in normal cerebrospinal fluid consist of albumin and globulin, the albumin being, as a general rule, in excess of the globulin, the greater portion of the globulin being pseudoglobulin. Euglobulin may also be found in normal cerebrospinal fluid and is usually increased in amount in diseased conditions. In pathologic states, both albumin and globulin are increased, the globulin being increased more in proportion than is albumin.

Thus Hewitt found that the ratio of albumin to globulin in normal spinal fluid was 8 to 1; in tabes 3 to 1; and in paresis about 1.3 to 1. This observer noted that the smallness of the albumin to the globulin ratio follows the strength of the Wassermann reaction and the extent of the precipitation of the colloidal gold solution.

Lange assumed that in syphilitic and paretic affections, specific proteins occurred in the cerebrospinal fluid, and these abnormal proteins caused the various colloidal gold precipitation curves.

Felton, quoted by Hewitt,[3] is of the opinion that abnormal relative amounts of globulin and albumin in the fluid are responsible for the colloidal gold reaction.

Weston, Cruickshank, Mellanby and Anwyl-Davies[3] held that globulin occurred in syphilitic and paretic affections in abnormally large amounts. These findings were not, however, the result of accurate quantitative analyses.

Hewitt[3] found that the total protein increase alone was not the chief factor in paretic fluid, and that the precipitation of colloidal gold solution must be attributed to other conditions because of the fact that the cerebrospinal fluid of meningitis and other conditions have a high total protein content, but these do not give the characteristic precipitation of gold solution.

Greenfield and Carmichael[5] maintain that there is no pathologic human cerebrospinal fluid in which the amount of globulin exceeds that of albumin. According to these observers, the proportion of albumin to globulin in various diseases is as follows: acute meningitis and arteriosclerosis 12 to 1; paresis 7 to 3; spinal tumor 2 to 1.

Greenfield and Carmichael further state that of the three globulins, pseudoglobulin is found in normal fluid. In pathologic conditions euglobulin and fibrinogen make their appearance. These two globulins are found in alterations of the cerebrospinal fluid and are associated with the presence of various antibodies. For instance, the Wassermann reacting substances, as well as hemolysin, separate on dialysis along with the euglobulin fraction. Some of the precipitins may be found in the pseudoglobulin fraction. Complement is usually found in the fibrinogen fraction.

STATE OF PROTEINS IN SPINAL FLUID

Kafka[6] holds that the various proteins in cerebrospinal fluid are subject to the same physicochemical changes as are the proteins of the blood serum.

Robertson,[4] in speaking of the proteins of the blood serum, states that there is much reason for believing that they are not merely present therein as a mixture, but in the form of a chemical complex possessing a different physical and chemical property from those of the constituent proteins out of which it is built up. It is felt that this same property applies to the total protein complex as well as to the integral protein fractions of cerebrospinal fluid.

Fremont-Smith and Ayer[1] maintain that it is possible that the protein present in normal ventricular fluid comes through the chorioid plexus from the blood plasma. In addition, transudation through dilated meningeal vessels may be the source of the proteins. There is therefore reason for the opinion that the various proteins of the cerebrospinal fluid have physical and chemical properties similar to those of the proteins of the blood.

PROTEIN CONTENT VALUES OF SPINAL FLUID

Ayer and Foster[2] observed that as the result of quantitative protein examinations of spinal fluid, some of the values which at first were believed to be normal were finally considered definite increases. They also noted that by means of quantitation, the border-line between normal and pathologic amounts of protein is more closely drawn.

These observers found that by means of quantitative estimations of the total protein of cerebrospinal fluid, the progress of treatment could be gauged in a manner similar to the study of the cell count, the Wassermann test, or the colloidal gold test. In fact, they noted that the total protein content of the cerebrospinal fluid began to fall early as the result of treatment in most diseases of the nervous system, except in paresis.

Ayer and Foster, in their investigation, found that the amount of protein

in normal spinal fluid lies between 16 mg. and 38 mg. per 100 c.c., and that more than 40 mg. is suspicious of an underlying diseased condition.

Fremont-Smith and Ayer[1] observed that in many border-line cases the total protein of cerebrospinal fluid may be more than doubled and yet the globulin test is negative. This indicates the need of exact methods for the estimation of albumin and globulin of the cerebrospinal fluid. They found that the amount of protein normally present in the lumbar fluid varied between 15 mg. and 45 mg. per 100 c.c.

Many observers have found that in the treatment of paresis the total protein may remain elevated after all other laboratory tests of the cerebrospinal fluid have become normal.

Mestrezat considered 15 mg. to 30 mg. of total protein as normal, while values over 35 mg. were considered pathologic. Eskuchen considered 20 mg. to 30 mg. as normal.

Some observers have found that in many fluids in which the protein content is normal, the Wassermann test is positive. This may be explained by the fact that the Wassermann reacting substances are present in euglobulin and this fraction may be increased without there being an increase of the total protein content of the fluid.

Hewitt[3] found that 30 mg. per 100 c.c. was the normal quantity of total protein. This observer found that the normal albumin content was about 20 mg. per 100 c.c. Globulin was present to the extent of 3 mg. per 100 c.c. Euglobulin was found to the extent of 0.5 mg. per 100 c.c.

QUANTITATIVE DETERMINATION OF PROTEINS OF SPINAL FLUID

Ayer,[2] in collaboration with Denis, elaborated a method for the estimation of total protein which has proved fairly satisfactory and not too difficult of application. This method consists of precipitating the cerebrospinal fluid proteins in a colloidal state by means of sulphosalicylic acid and reading the turbidity in the unknown by means of a colorimeter against a standard solution prepared at the same time from a blood serum of known content.

According to this technic the amount of protein in normal spinal fluid lies between 16 mg. and 38 mg. per 100 c.c. More than 40 mg. is considered suspicious of an underlying pathologic process.

Young and Bennett[7] have devised a method which consists of precipitating the total protein of cerebrospinal fluid by alcohol, acetic acid and heat, then measuring the resultant precipitate volumetrically in capillary-tipped vaccine tubes and reading it in terms of milligrams per 100 c.c. According to this method, normal readings of total protein vary from 25 mg. to 75 mg. per 100 c.c.

H. Wu[8] made use of the color reaction of proteins of the blood with phospho-18-molybdic tungstic acid (phenol reagent). The proteins of the blood serum were precipitated and separated from each other. Phenol reagent was then added to bring about a color reaction, the color being compared with a standard tyrosine solution. The color of the protein obtained upon the addition of the phenol reagent is due to its tyrosine content and is constant for any given protein.

In 1926, Ling[9] applied Wu's colorimetric method for the estimation of proteins of blood serum to spinal fluid and determined the total protein of a small number of specimens of fluid. No attempt was made by Ling to determine the separate protein fractions.

Hewitt[3] elaborated colorimetric methods for the determination of the total protein, and the various protein fractions of cerebrospinal fluid, based upon the method of Wu with blood serum.

The method consists in precipitating the globulin from the fluid by the addition of an appropriate amount of ammonium sulphate and the determination of the protein content of the original fluid and in the filtrates from the ammonium sulphate precipitation. This is done by precipitation of the proteins by tungstic acid and measuring the color produced by the precipitates with phenol reagent.

MODIFIED PROCEDURES FOR THE DETERMINATION OF SPINAL FLUID PROTEINS

The methods for the determination of albumin, globulin and total protein of the cerebrospinal fluid described herein are based upon the work of Hewitt[3] and Wu.[8] According to our procedure, individual technics are used for the quantitative estimation of globulin and total protein. The albumin content is obtained by deducting the globulin from the amount of total protein.

Wu[8] found the tyrosine equivalents of globulin and albumin to be as follows:

1 mg. of tyrosine equals { 25.2 mg. globulin / 27.5 mg. albumin

Reagents used in modified procedure:

1. Tyrosine solution
2. Standard solution of tyrosine (weak)
3. Standard solution of tyrosine (strong)
4. Phenol reagent
5. 20 per cent sodium carbonate
6. 1 per cent sodium hydrate

TYROSINE SOLUTION

Dissolve 50 mg. tyrosine (Pfannenstiel) in 250 c.c. of N/10 HCl. This solution usually does not deteriorate after standing for a period of six to eight months.

WEAK AND STRONG TYROSINE STANDARDS

It has been found that, in order to make colorimetric comparisons, two standards should be prepared simultaneously with the unknown. A weak standard containing 0.1 mg. tyrosine and a strong standard containing 0.2 mg. tyrosine. The particular standard to be used in the determination depends upon which of the two standards the color of the unknown solution approximates.

The tyrosine standard solutions are made up as follows:

1. Into one of two 25 c.c. glass stoppered cylinders place 1 c.c. tyrosine solution containing 0.2 mg. tyrosine (strong standard); into the other, place 0.5 c.c. tyrosine solution containing 0.1 mg. tyrosine (weak standard).
2. Add to each cylinder: 0.25 c.c. phenol reagent and distilled water up to 20 c.c.
3. Add 2.5 c.c. 20 per cent sodium carbonate.
4. Add distilled water up to 25 c.c.

PHENOL REAGENT

1. Into a clean 1000 c.c. pyrex beaker, place 100 gm. sodium tungstate, 20 gm. phosphomolybdic acid, 50 c.c. phosphoric acid (85 per cent) and 750 c.c. distilled water.

2. Boil mixture continuously for two hours.

3. Cool at room temperature and dilute with distilled water to 1000 c.c. Filter if necessary. The final product should be of a brilliant canary yellow color.

TECHNIC FOR GLOBULIN DETERMINATION*

1. Place 5 c.c. cerebrospinal fluid in a 15 c.c. centrifuge tube and add 5 c.c. chemically pure saturated solution ammonium sulphate.

2. Place tube in water-bath at 56° C. until maximum precipitation occurs. This usually takes about thirty minutes.

3. Centrifuge at high speed for about ten minutes until the globulin precipitate becomes firmly packed at bottom of tube. Pour off supernatant fluid completely and drain the tube thoroughly by inverting. Remove any of the ammonium sulphate at the mouth of the tube with filter paper.

4. Add 1 c.c. of 10 per cent sodium tungstate and shake bottom of tube until the globulin precipitate is dissolved.

5. Remove this alkaline globulin solution with a pipette into another clean 15 c.c. centrifuge tube, taking care that the pipette does not touch the walls of the tube, and that no ammonium sulphate is carried over.

6. Add 4 c.c. distilled water and 1 c.c. 2/3 N. sulphuric acid. The globulin is thus again precipitated.

7. Centrifuge for five minutes and discard the supernatant fluid.

8. Add 2 or 3 drops of 1 per cent sodium hydroxide and shake bottom of tube to help dissolve globulin precipitate.

9. Add 2 c.c. distilled water and 0.1 c.c. phenol reagent.

10. Add 1 c.c. 20 per cent sodium carbonate.

11. Add distilled water to a volume of 10 c.c. and set tube aside for fifteen minutes for maximum blue color to develop.

12. Compare clear, blue solution with weak or strong tyrosine standard prepared simultaneously.

GENERAL FORMULA FOR GLOBULIN OR TOTAL PROTEIN CALCULATION

$$\frac{S}{U} \times SS \times \frac{Vu}{Vs} \times \frac{100}{X} = \text{mg. per 100 c.c. cerebrospinal fluid.}$$

Explanation of formula:

$$\frac{S}{U} = \frac{\text{Reading of standard}}{\text{Reading of unknown}}$$

Note: The fraction $\frac{S}{U}$ varies depending upon the reading of the standard and the reading of the unknown. Ordinarily the standard is set at 20. The denominator of the fraction $\frac{S}{U}$ indicates the reading of the unknown, which is variable.

SS = Strength of standard (in mg. per c.c.)

Note: The SS factor varies with the type of standard used. If the strong standard is used, SS equals 0.2 mg. If the weak standard is used, SS equals 0.1 mg.

$$\frac{Vu}{Vs} = \frac{\text{Volume of unknown}}{\text{Volume of standard}}$$

Note: This fraction is constant. The unknown, in each instance, being brought up to a volume of 10 c.c. and the standard being brought up to a volume of 25 c.c. Therefore $\frac{Vu}{Vs} = \frac{10}{25}$.

$$\frac{100}{X} = \frac{\text{Per 100 c.c. cerebrospinal fluid}}{\text{Amount of cerebrospinal fluid used in the determination}}$$

Note: The denominator of the fraction $\frac{100}{X}$ varies depending upon the amount of cerebrospinal fluid used in the determination.

*If unknown solution is turbid, centrifugate and transfer supernatant fluid to colorimeter cup.

APPLICATION OF GENERAL FORMULA TO ESTIMATE QUANTITY OF GLOBULIN

Five c.c. cerebrospinal fluid was used in the determination of globulin. The strong standard was used, the colorimeter was set at 20, and the reading of the unknown was 16, therefore:

$$\frac{20}{16} \times 0.2 \times \frac{10}{25} \times \frac{100}{5} = 2 \text{ mg. tyrosine-globulin per 100 c.c.}$$

Since 1 mg. tyrosine equals 25.2 mg. globulin, then:

$$25.2 \times 2 = 50.4 \text{ mg. globulin per 100 c.c.}$$

TOTAL PROTEIN DETERMINATION

1. Place 2 c.c. cerebrospinal fluid in a 15 c.c. graduated centrifuge tube, add 1 c.c. 10 per cent sodium tungstate and 1 c.c. 2/3 N. sulphuric acid. Mix thoroughly and set aside at room temperature for thirty minutes, or until maximum precipitation of the proteins takes place.
2. Centrifuge at high speed for about 10 minutes.
3. Pour off supernatant fluid.
4. Add 2 or 3 drops of 1 per cent sodium hydroxide and shake bottom of tube.
5. Add 3 c.c. distilled water and 0.1 c.c. phenol reagent.
6. Add 1 c.c. 20 per cent sodium carbonate solution.
7. Add distilled water to a volume of 10 c.c. and allow to stand for fifteen minutes, until maximum blue color develops.
8. Compare the unknown with weak or strong standard as in globulin determination.

APPLICATION OF GENERAL FORMULA TO ESTIMATE QUANTITY OF TYROSINE-TOTAL PROTEIN

Two c.c. cerebrospinal fluid was used in the determination of total protein. The strong standard was used; the colorimeter was set at 20; and the reading of the unknown was 15; therefore:

$$\frac{20}{15} \times 0.2 \times \frac{10}{25} \times \frac{100}{2} = 5.33 \text{ mg. tyrosine-total protein per 100 c.c.}$$

DETERMINATION AND COMPUTATION OF ALBUMIN

In order to estimate the amount of albumin in cerebrospinal fluid, it is necessary to subtract the tyrosine-globulin from the tyrosine-total protein.

In the foregoing example it is as follows:

$$5.33 - 2 = 3.33 \text{ mg. tyrosine-albumin per 100 c.c.}$$

Since the tyrosine equivalent for albumin is 27.5, then:

$$27.5 \times 3.33 = 91.6 \text{ mg. albumin per 100 c.c.}$$

MANNER OF ESTIMATING AMOUNT OF TOTAL PROTEIN

In order to estimate the amount of total protein it is necessary to add the albumin and globulin, therefore:

$$91.6 + 50.4 = 142 \text{ mg. total protein per 100 c.c.}$$

TECHNICAL DIFFICULTIES

In using this method, turbid solutions are frequently obtained, the cause of which is either chemical or technical. Generally, the turbidity is due to the presence of sodium salts, chiefly carbonates, and is the result of the addition of 20 per cent sodium carbonate, which is used to dissolve the protein precipitate. In the technic described herein, 2 or 3 drops of 1 per cent sodium hydroxide are used initially in dissolving the protein precipitate; this tends to eliminate turbidity.

Another cause of turbidity in the unknown solution is the incomplete removal of ammonium sulphate used to precipitate the globulins. It is therefore important to remove this salt as completely as possible so that the final solution is of a clear blue color.

Both the unknown and the standard solutions should be prepared simultaneously and allowed to stand for the same length of time, so that the maximum development of blue color will take place in both solutions.

GENERAL CONSIDERATIONS

During the conduct of this investigation it was our aim to ascertain the following points:

1. The total protein, globulin and albumin of normal cerebrospinal fluid, using this modified procedure, also the variations in such diseases as paresis, tabes, tertiary syphilis, multiple sclerosis, meningitis, etc.

2. The ratio of albumin to globulin in normal spinal fluid, as well as in pathologic fluids.

3. The relationship of the cerebrospinal fluid proteins to the colloidal gold test.

4. The effect of treatment upon the individual protein fractions of cerebrospinal fluid, as well as upon the total protein.

THE PROTEINS IN NONMENINGITIC AND NONSYPHILITIC CEREBROSPINAL FLUIDS

On account of the inability to obtain cerebrospinal fluid from strictly normal subjects, it was necessary to use specimens from subjects under treatment for various miscellaneous conditions which are ordinarily not accompanied by abnormal protein contents.

Table I shows that, in a series of 10 nonsyphilitic and nonmeningitic cerebrospinal fluids, the albumin content ranged from 21 mg. to 52 mg. per 100 c.c., the average being 34 mg.

In the same series the globulin content ranged from 6 mg. to 20 mg. per 100 c.c., the average being 13 mg.

The total protein ranged from 27 mg. to 62 mg. per 100 c.c., the average being 48 mg.

The ratio of albumin to globulin in the 10 cases studied ranged from a high of 5.2:1 to a low of 1.4:1, the average ratio of albumin to globulin being 2.6:1.

TABLE I

PROTEINS OF CEREBROSPINAL FLUID IN NONMENINGITIC AND NONSYPHILITIC DISEASES

NUMBER	MG. PER 100 C.C.			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE	DIAGNOSIS
	ALBUMIN	GLOBULIN	TOTAL PROTEIN			
1	25	18	43	1.4:1	0001100000	Aortic aneurysm
2	30	15	45	2.0:1	0011000000	Aortic insufficiency
3	45	13	58	3.5:1	0011100000	Chronic alcoholism
4	42	12	54	3.5:1	0011100000	Dementia precox
5	26	12	38	2.2:1	1112221000	Influenza
6	40	12	52	3.3:1	1112210000	Constitutional psychopathic state
7	30	14	44	2.1:1	0011000000	Dementia precox
8	33	20	53	1.7:1	0011210000	Hypomania
9	52	10	62	5.2:1	1112210000	Manic-depressive psychosis
10	21	6	27	3.5:1	1112210000	Hysteria

PROTEINS OF CEREBROSPINAL FLUID IN UNTREATED CASES OF PARESIS

In reviewing the data of Table II it is noted that in the examination of the proteins of 16 specimens of cerebrospinal fluid from cases of paresis, the albumin content ranged from 35 mg. to 160 mg. per 100 c.c., the average being 65 mg.

The globulin content ranged from 21 mg. to 93 mg. per 100 c.c., the average being 44 mg.

The total protein content ranged from 63 mg. to 204 mg. per 100 c.c., the average being 110 mg.

The ratio of albumin to globulin varied from a high of 3.6:1 to a low of 0.6:1, the average being 1.5:1.

In normal cerebrospinal fluid the amount of albumin invariably exceeds the amount of globulin. It is noted that in the untreated cases of paresis reported in Table II the amount of globulin is almost equal to that of albumin, in fact, in three of the specimens reported in Table II the quantity of globulin exceeded the quantity of albumin (Cases 1, 4, and 16).

TABLE II

CEREBROSPINAL FLUID PROTEINS IN UNTREATED CASES OF PARESIS

NUMBER	MG. PER 100 C.C.			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE
	ALBUMIN	GLOBULIN	TOTAL PROTEIN		
1	68	93	161	0.7:1	5555555331
2	82	33	115	2.5:1	5555532100
3	49	32	81	1.5:1	5555552100
4	42	69	111	0.6:1	5555543210
5	42	21	63	2.0:1	5555544310
6	58	27	85	2.1:1	5555443100
7	82	69	151	1.2:1	1224333210
8	67	25	92	2.7:1	5555553210
9	84	45	129	1.9:1	5555555331
10	35	30	65	1.2:1	5555432100
11	57	47	104	1.2:1	5555553210
12	55	45	100	1.2:1	5555554431
13	60	43	103	1.4:1	5555553210
14	59	28	87	2.1:1	5555553210
15	160	44	204	3.6:1	5555554210
16	44	60	104	0.7:1	5555534210

PROTEINS OF CEREBROSPINAL FLUID IN TABES DORSALIS

The quantitative estimation of the total protein, as well as of the albumin and globulin contents of cerebrospinal fluid in 20 specimens of fluid from cases of tabes shows that the albumin content ranged from 23 mg. to 63 mg. per 100 c.c., the average being 42 mg.

The globulin content ranged from 8 mg. to 46 mg. per 100 c.c., the average being 20 mg.

The total protein content ranged from 39 mg. to 87 mg. per 100 c.c., the average being 62 mg.

The ratio of albumin to globulin varied from a high of 6.8:1 to a low of 0.5:1, the average being 2.1:1.

TABLE III

CEREBROSPINAL FLUID PROTEINS IN TABES DORSALIS

NUMBER	MG. PER 100 C.C.			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE
	ALBUMIN	GLOBULIN	TOTAL PROTEIN		
1	49	14	63	3.5:1	0011100000
2	49	14	63	3.5:1	0011100000
3	54	8	62	6.8:1	0011100000
4	23	26	49	0.9:1	1122100000
5	53	13	66	4.1:1	1122221000
6	52	15	67	3.5:1	1122210000
7	63	16	79	4.0:1	1122332100
8	43	9	52	4.8:1	1112221000
9	25	46	71	0.5:1	1223332100
10	23	31	54	0.7:1	1234432100
11	41	16	57	2.6:1	1122100000
12	50	12	62	4.2:1	1233321000
13	48	18	66	2.7:1	1112221000
14	38	15	53	2.5:1	1112210000
15	39	35	74	1.1:1	1123321000
16	31	26	57	1.2:1	1123321000
17	47	40	87	1.2:1	1234432100
18	60	15	75	4.0:1	1223321000
19	24	15	39	1.6:1	0011110000
20	28	16	44	1.8:1	0011100000

PROTEINS OF CEREBROSPINAL FLUID IN TERTIARY SYPHILIS

Table IV lists the results of 31 determinations of cerebrospinal fluid of cases of tertiary syphilis. It is noted that the albumin content ranged from 25 mg. to 79 mg. per 100 c.c., the average being 45 mg.

The globulin ranged from 8 mg. to 25 mg. per 100 c.c., the average being 16 mg.

The total protein ranged from 37 mg. to 104 mg. per 100 c.c., the average being 61 mg.

The ratio of albumin to globulin varied from a high of 7.6:1 to a low of 1.4:1, the average being 2.9:1.

It is noted that the quantities of the various proteins of cerebrospinal fluid of cases of tertiary syphilis are in excess of those in nonmeningitic and nonsyphilitic conditions. Whether or not tertiary syphilis is accompanied by an involvement of the central nervous system with a concomitant increase of the proteins of the cerebrospinal fluid cannot be definitely stated at this time, however, the data in Table IV would lead one to infer that this is a possibility.

PROTEINS OF CEREBROSPINAL FLUID IN MULTIPLE SCLEROSIS

In the course of this study estimations were made of the albumin, globulin and total protein contents of cerebrospinal fluid of a number of cases of multiple sclerosis, with a view of ascertaining whether or not the quantities of these fractions were comparable with those of paretic cerebrospinal fluid, the object being to determine the reason for the similarity of the colloidal gold curves in these two conditions.

Table V reveals the fact that the albumin content varied from 43 mg. to 117 mg. per 100 c.c., the average being 71 mg. In paresis, the range was from 35 mg. to 160 mg. per 100 c.c., the average being 65 mg.

TABLE IV

CEREBROSPINAL FLUID PROTEINS IN TERTIARY SYPHILIS

NUMBER	MG. PER 100 C.C.			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE
	ALBUMIN	GLOBULIN	TOTAL PROTEIN		
1	43	22	65	2.0:1	1122210000
2	59	12	71	4.9:1	0012310000
3	35	25	60	1.4:1	1122210000
4	40	11	51	3.6:1	1233321000
5	32	10	42	3.2:1	0122100000
6	41	20	61	2.1:1	1233321000
7	48	22	70	2.2:1	1123321000
8	42	16	58	2.6:1	1123310000
9	58	18	76	3.2:1	0011210000
10	29	19	48	1.5:1	0001100000
11	49	20	69	2.5:1	1112221000
12	35	12	47	2.9:1	1122100000
13	60	15	75	4.0:1	1122333210
14	28	13	41	2.2:1	1123321000
15	59	15	74	3.9:1	1123332100
16	36	8	44	4.5:1	1122221000
17	45	13	58	3.5:1	0011100000
18	37	19	56	1.9:1	1123321000
19	31	14	45	2.2:1	1122210000
20	39	15	54	2.6:1	1122100000
21	74	22	96	3.4:1	1122321000
22	34	20	54	1.7:1	2233321000
23	40	16	56	2.5:1	1122210000
24	79	25	104	3.2:1	2233331000
25	25	12	37	2.1:1	0011100000
26	76	10	86	7.6:1	1122210000
27	38	11	49	3.5:1	1122100000
28	47	14	61	3.4:1	0012221000
29	44	13	57	3.4:1	0012100000
30	55	12	67	4.6:1	1112221000
31	52	11	63	4.7:1	1112221000

The globulin content varied from 20 mg. to 49 mg. per 100 c.c., the average being 33 mg. In paresis the globulin range was from 21 mg. to 93 mg. per 100 c.c., the average being 44 mg.

The total protein content varied from 66 mg. to 140 mg. per 100 c.c., the average being 104 mg. In paresis the range was from 63 mg. to 204 mg. per 100 c.c., the average being 110 mg.

The ratio of albumin to globulin varied from 5.3:1 to 1.4:1, the average being 2.2:1. In paresis the ratio varied from 3.6:1 to 0.6:1, the average being 1.5:1.

The above findings indicate that there is a slight similarity of the quantities of the protein fractions in paresis and multiple sclerosis. Whether or not this accounts for the type of colloidal gold reaction in these two conditions cannot be definitely stated, inasmuch as the nature and the mechanism of this reaction are not thoroughly understood.

THE PROTEINS OF CEREBROSPINAL FLUID DURING THE TREATMENT OF PARESIS

For the purpose of ascertaining whether or not antisyphilitic treatment has any effect on the albumin, globulin, and total protein contents, determinations were made on a series of cases while receiving treatment.

TABLE V

CEREBROSPINAL FLUID PROTEINS IN MULTIPLE SCLEROSIS

CASE NUMBER	DATE	MG. PER 100 C.C.			RATIO OF ALBUMIN TO GLOBULIN
		ALBUMIN	GLOBULIN	TOTAL PROTEIN	
1	10/27/27	91	49	140	1.9:1
	5/21/28	117	22	139	5.3:1
	7/13/28	72	43	115	1.7:1
2	7/13/28	43	23	66	1.9:1
3	7/13/28	48	20	68	2.4:1
4	7/13/28	56	39	95	1.4:1

The findings may be seen by referring to Table VI. The albumin, globulin and total protein of cerebrospinal fluid of 21 cases of paresis were studied, and it was found that the albumin content was reduced in 17, or 81 per cent, of the cases; the globulin content was reduced in 13, or 62 per cent; and the total protein was reduced in 17, or 81 per cent, of the cases during the treatment régime.

It is interesting to note that, during this period, the ratio of albumin to globulin was reduced in 12, or 57 per cent, of the cases; an increase of the ratio was observed in 8 cases; and in 1 case no change was noted.

The above may be explained by the fact that treatment caused a reduction of the albumin content of spinal fluid more often and more readily than the globulin content, with the result that the ratio of albumin to globulin was reduced more frequently than it was increased.

THE AVERAGE PROTEIN CONSTITUENTS IN VARIOUS DISEASES

In reviewing Table VII it is noted that the smallest quantities of the various proteins are to be found in the nonmeningitic and nonsyphilitic diseases.

A slight increase of the proteins was found in the cerebrospinal fluid of cases of tertiary syphilis.

The largest quantities of the various proteins are found in paresis and multiple sclerosis.

The ratio of albumin to globulin is high in the nonmeningitic and nonsyphilitic fluids. The lowest ratio of albumin to globulin was found in cerebrospinal fluids of paresis.

THE RELATIONSHIP OF THE CEREBROSPINAL FLUID PROTEINS TO THE COLLOIDAL GOLD REACTION

One of the purposes of this study was to ascertain whether or not changes of the protein quantities, or changes in the quantitative relation of albumin to globulin were in any way responsible for the various characteristic colloidal gold curves.

J. Cruickshank[10] has observed that the globulin obtained from normal spinal fluid, even when used in concentrated form, is almost inactive when added to a solution of colloidal gold. He, therefore, is of the opinion that the precipitating action of paretic fluid cannot be ascribed to this protein fraction alone, but is probably dependent on a specific alteration of the physical state

TABLE VI
PROTEINS OF CEREBROSPINAL FLUID IN PARESIS DURING TREATMENT

CASE NUMBER	DATE	MG. PER 100 C.C.			RATIO OF ALBUMIN TO GLOBULIN	COLLOIDAL GOLD CURVE	TREATMENT
		ALBUMIN	GLOBULIN	TOTAL PROTEIN			
1	10/26/27	55	15	70	3.7:1	5555553200	Tryparsamide
	1/ 9/28	57	27	84	2.1:1	4433321000	and
	3/21/28	68	27	95	2.5:1	1233321000	Mercury
2	10/25/27	41	32	73	1.3:1	5555544321	Tryparsamide
	1/ 5/28	72	40	112	1.8:1	5554431000	and
	3/19/28	35	17	52	2.1:1	5554443100	Mercury
3	11/ 7/27	84	49	133	1.7:1	2233321000	Tryparsamide
	2/ 2/28	102	37	139	2.8:1	2223333100	and
	4/19/28	104	22	126	4.7:1	1233544210	Mercury
4	11/23/27	56	21	77	2.7:1	1233331000	Tryparsamide
	2/ 6/28	49	20	69	2.5:1	2233321000	Mercury and
	4/30/28	35	30	65	1.2:1	1233321000	Bismuth
5	12/ 1/27	49	76	125	0.6:1	5555555210	Malaria Inocula-
	1/19/28	49	44	93	1.1:1		tions, Tryparsa-
	3/26/28	33	32	65	1.0:1	3555443210	mide and Mercury
6	12/ 5/27	44	27	71	1.6:1	5555532100	Tryparsamide
	2/20/28	40	22	62	1.8:1	5555543210	and
	4/23/28	35	23	58	1.5:1	2345531000	Mercury
7	1/ 3/28	66	40	106	1.7:1	1112221000	Tryparsamide
	3/15/28	61	24	85	2.5:1	3343321000	and
	5/28/28	65	26	91	2.5:1	5333441000	Mercury
8	10/26/27	82	37	119	2.2:1	5555544321	Tryparsamide
	3/19/28	63	22	85	2.9:1	5555543210	and
	5/31/28	56	19	75	2.9:1	1233331000	Mercury
9	11/ 4/27	38	15	53	2.5:1	1233310000	Tryparsamide
	5/ 5/28	41	17	58	2.4:1	1123332100	and Mercury
10	11/28/27	69	32	111	2.2:1	5555554210	Tryparsamide
	4/26/28	46	33	79	1.4:1	3444432100	and
	7/27/28	49	37	86	1.3:1	2554332100	Bismuth
11	11/28/27	47	23	70	2.0:1	5555432100	Tryparsamide
	2/ 9/28	39	19	58	2.1:1	2233210000	and Mercury
12	12/ 5/27	78	33	111	2.4:1	5555554210	Tryparsamide
	3/ 1/28	40	30	70	1.3:1	2443431000	and Mercury
13	11/ 7/27	44	29	73	1.5:1	1122331000	Tryparsamide
	4/ 2/28	29	19	48	1.5:1	2223331000	and Mercury
14	12/ 5/27	72	35	107	2.1:1	5555554210	Tryparsamide
	2/27/28	46	26	72	1.8:1	2235432100	and Mercury
15	12/ 8/27	50	21	71	2.4:1	5555543100	Sodium Iodide
	2/ 3/28	47	21	68	2.2:1	5554321000	and Mercury
16	12/13/27	36	19	55	1.9:1	5554432100	Tryparsamide
	3/ 5/28	30	17	47	1.8:1	2233321000	and Mercury
17	12/19/27	44	14	58	3.1:1	1112221000	Sulpharsphenamine
	3/ 1/28	38	20	58	1.9:1	1112221000	and Mercury
18	12/19/27	56	19	75	2.9:1	1112332100	Tryparsamide
	3/ 1/28	38	20	58	1.9:1	1122333100	and Mercury
19	3/ 8/28	48	35	83	1.4:1	5555543100	Tryparsamide
	5/21/28	37	29	66	1.3:1	5555553100	and
	8/ 1/28	29	50	79	0.6:1	5555532000	Mercury
20	1/ 5/28	51	23	74	2.2:1	2233210000	Tryparsamide
	5/31/28	38	15	53	2.5:1	2333321000	and Mercury
21	10/26/27	57	22	79	2.6:1	1233321000	Tryparsamide
	3/29/28	63	22	85	2.9:1	1123431000	and
	7/16/28	76	17	93	4.5:1	1122310000	Mercury

TABLE VII

SHOWING THE AVERAGE PROTEIN CONSTITUENTS ALSO ALBUMIN-GLOBULIN RATIO IN VARIOUS DISEASES

	AVERAGE ALBUMIN	AVERAGE GLOBULIN	AVERAGE TOTAL PROTEIN	AVERAGE RATIO ALBUMIN TO GLOBULIN
Non-meningitic and non-syphilitic diseases	34	13	48	2.6:1
Tertiary syphilis	45	16	61	2.9:1
Tabes dorsalis	42	20	62	2.1:1
Paresis	65	44	110	1.5:1
Multiple sclerosis	71	33	104	2.2:1

of the globulin which is associated with an increased electric charge. Cruickshank further maintains that this alteration of the globulin cannot be regarded as specific for syphilis since it may occur also in multiple sclerosis. He states that the phenomenon of colloidal gold reaction is dependent upon the albumin and globulin contents of the fluid, the precipitation of the gold being due to the globulin and its retention in solution being due to the albumin fraction.

Mellanby and Anwyl-Davies,[11] as a result of their studies, established the hypothesis that the precipitating factors of the colloidal gold solution were either euglobulin or pseudoglobulin, or a combination of these. The euglobulin being the active agent which precipitates colloidal gold, and the pseudoglobulin fraction tends to keep the gold in solution and antagonizes the precipitating action of the euglobulin. These observers are of the opinion that all cerebrospinal fluids contain a constant quantity of pseudoglobulin but variable quantities of euglobulin, and that the different colloidal gold reactions are due to the varying quantities of euglobulin.

If this assumption is correct, then the characteristic precipitation of the gold solution obtained in paresis and multiple sclerosis should not persist after treatment when the globulin fraction is diminished in amount and the ratio of albumin to globulin is increased.

The data in Table VI shows that the characteristic colloidal gold precipitation is not due entirely to the relative quantities of globulin and albumin in the cerebrospinal fluid because instances are seen where the globulin is decreased and albumin increased, and yet the typical paretic curve persists. Furthermore, in reviewing the findings of Case No. 1 of Table V, in which there were three separate estimations of the protein fractions, the examination of October 27, 1927, in which the ratio of albumin to globulin was 1.9:1, was accompanied by a typical paretic curve (5555543210); while the examination of July 13, 1928, in which the ratio of albumin to globulin was similar (1.7:1), was accompanied by a diminished precipitation of the gold (2233310000).

Unfortunately, a study was not made of the relative quantities of euglobulin and pseudoglobulin fractions of the cerebrospinal fluid in various diseases, so that it is impossible to confirm the hypothesis of Mellanby and Anwyl-Davies that variations of the euglobulin fraction caused the various characteristic colloidal gold curves.

It may therefore be concluded that there is some phenomenon other than the quantities of albumin and globulin or the relative proportions of albumin to globulin which is responsible for the characteristic colloidal gold precipitation reactions in the various diseases. Whether the precipitation of the colloidal gold solution is dependent upon a physical change of the globulin, i.e., upon an increased electric charge, as maintained by Cruickshank, or is dependent upon the amount of euglobulin in the fluid cannot be stated at this time.

SUMMARY AND CONCLUSIONS

1. On account of the importance of the proteins of the cerebrospinal fluid as an aid to diagnosis, as well as for the guidance of treatment, it is felt that the quantitative determination of these constituents should be done routinely.

2. Accordingly, the method of Wu for the estimation of the blood proteins and that of Hewitt for spinal fluid proteins have been modified and a colorimetric technic has been developed.

3. By this modified procedure the quantities of globulin and total protein are estimated and the albumin is determined by deducting the amount of globulin from the total protein.

4. a. The average quantities per 100 c.c. of the various proteins of nonmeningitic and nonsyphilitic cerebrospinal fluids were: albumin, 34 mg.; globulin, 13 mg.; total protein, 48 mg. The average ratio of albumin to globulin was 2.6:1.

b. The average quantities per 100 c.c. of the various proteins of spinal fluid in untreated paretics were: albumin, 65 mg.; globulin, 44 mg.; total protein, 110 mg. The average ratio of albumin to globulin was 1.5:1.

c. The average quantities per 100 c.c. of the various proteins of spinal fluid in tabes dorsalis were: albumin, 42 mg.; globulin, 20 mg.; total protein, 62 mg. The average ratio of albumin to globulin was 2.1:1.

d. The average quantities per 100 c.c. fluid of the various proteins of cases of tertiary syphilis were: albumin, 45 mg.; globulin, 16 mg.; total protein, 61 mg. The average ratio of albumin to globulin was 2.9:1.

It is noted that the quantities of the various proteins of cerebrospinal fluids of tertiary syphilis without apparent involvement of the central nervous system were slightly in excess of the quantities of the proteins in nonmeningitic and nonsyphilitic fluids.

e. The average quantities of the various proteins per 100 c.c. fluid in cases of multiple sclerosis were: albumin, 71 mg.; globulin, 33 mg.; total protein, 104 mg. The average ratio of albumin to globulin was 2.2:1.

It is noted that the quantities of the different protein fractions in this condition are similar to those of paresis. It is possible that the type of colloidal gold curve found in these two conditions is due to the similarity of the quantities of the protein fractions. Inasmuch as the quantitative estimation of the euglobulin fraction was not made, it was not possible to confirm the hypothesis of Mellanby and Anwyl-Davies that variations of the euglobulin fraction cause the characteristic colloidal gold curves.

5. A study was made of the proteins of the cerebrospinal fluid of a number of cases of paresis while undergoing treatment. It was found that during treatment a reduction of the albumin fraction took place. The globulin content was not influenced as readily nor as frequently as was the albumin. The ratio of albumin to globulin varied, in more than half of the cases the ratio was decreased during treatment.

Improvement should be followed by an increase of the albumin to globulin ratio. The decrease was evidently due to the fact that the albumin fraction was more greatly influenced by treatment than the globulin fraction.

6. The smallest quantities of the various proteins were found in the nonmeningitic and nonsyphilitic fluids. The largest quantities of the various proteins were found in paresis and multiple sclerosis. The ratio of albumin to globulin was high in the nonmeningitic and nonsyphilitic fluids. The lowest ratio of albumin to globulin was found in paresis.

REFERENCES

1. Fremont-Smith, Frank, and Ayer, James B.: The Association for Research in Nervous Disease. The Human Cerebrospinal Fluid, 1926, N. Y., Paul B. Hoeber.
2. Ayer, James B., and Foster, Harold E.: Quantitative Estimation of the Total Protein in the Cerebrospinal Fluid, J. A. M. A. **77**: 365-369, 1921.
3. Hewitt, Leslie Frank: Proteins of the Cerebrospinal Fluid, Brit. J. Exper. Path. **8**: 84-92, 1927.
4. Robertson, T. Brailsford: Physical Chemistry of the Proteins, 1918, Longmans.
5. Greenfield, J. G., and Carmichael, E. A.: The Cerebrospinal Fluid in Clinical Diagnosis, London, 1925, Macmillan & Co.
6. Kafka, V.: Die Eiweissrelation des Liquor cerebrospinalis, Ztschr. f. d. ges. Neural. u. Psych. **106**: 54-69, 1926.
7. Young, G. Alexander, and Bennett, A. E.: Studies on the Quantitative Estimation of the Total Protein Content in Cerebrospinal Fluid, Am. J. Med. Sc. **172**: 249-255, 1926.
8. Wu, Hsien: A New Colorimetric Method for the Determination of Plasma Proteins, J. Biol. Chem. **51**: 33-39, 1922.
9. Ling, S. M.: The Determination of Protein in Spinal Fluid, With a Note on the Increase in Protein in the Spinal Fluid in Typhus Fever, J. Biol. Chem. **69**: 397-401, 1926.
10. Cruickshank, J.: The Value and Mechanism of the Colloidal Gold Test, Brit. J. Exper. Path. **1**: 71-88, 1920.
11. Mellanby, John, and Anwyl-Davies, Thomas: The Precipitation of Colloidal Gold by Cerebrospinal Fluid; The Diagnosis of Neurosyphilis, Brit. J. Exper. Path. **4**: 132-145, 1923.

QUANTITATIVE MICROSCOPIC URINALYSIS*

By William G. Exton, M.D., Newark, N. J.

INTRODUCTION

In the course of a year I see hundreds of urinalysis reports from different laboratories throughout the United States and Canada, and it is interesting to note that all of them report their microscopic findings in terms which predicate the existence of quantitative methods of urinary microscopy. It is also noteworthy that our textbooks do not suggest that microscopic urinalysis is a quantitative procedure and that the literature touches on the subject only in connection with a few special studies which utilize modified blood counting technics to enumerate the microscopic objects in urine.

In our work, many specimens come from people who also send their urines to other laboratories, and the examinations bring to light numerous discrepancies which the interested laboratories must account for in order to appease the disturbed minds of physicians and patients. In such instances the undeniable alibi that the urine a person passes may vary from time to time usually gets by, by virtue of its silent intimation that the reports would have agreed had both laboratories examined portions of the same urine.

It also often happens in our experience, however, that different laboratories do examine portions of the same urine and nevertheless report similar discrepancies. In such instances alibis lose their smugness and ordinarily degenerate into one laboratory resting, as it were, on its findings while what may be called the "negative" laboratory sidesteps with the implication that some laboratories find pus or casts, as the case may be, in every urine. Unfortunately, such occurrences are frequent and acutely embarrassing because they create a situation which clinicians and laymen cannot understand without resorting to theories that discredit clinical pathology and pathologists.

The thinking laboratory man, of course, knows better. To paraphrase an old saying about the spirit being willing but the flesh weak, he knows that he reports his microscopic findings in terms which are conventional rather than actually quantitative, and those who have not considered the matter may learn from a few experiments that differences in technics do not account for all of the discrepancies because well-trained technicians who carry through a stereotyped technic in the same laboratory on portions of the same specimen of urine will report results that vary by hundreds of per cent.

Uranalyses are made so frequently and repeatedly that it seemed to me more than desirable to remove a situation which so often reproaches clinical

*From the Prudential Laboratory and Longevity Service, the Prudential Insurance Company of America, Newark, N. J.

Read before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.

pathology because of the perplexities and irritations it causes not only laboratory men and clinicians but also, and in increasing numbers nowadays, laymen.

The work here outlined was therefore undertaken with the object of improving technics so as to increase precision, because only in this way did a chance seem open to enable different laboratories to report microscopic findings with something like uniformity.

For the sake of clarity the material is divided into two parts. Part I reviews the methods currently practiced by different laboratories and the experimental checks which were made of the various manipulative details entering into the technics of urinary microscopy. Part II presents, step by step, the details of a practicable procedure for microscopic uranalysis which has experimentally and in practice proved accurate enough to enable reporting results with a uniformity satisfactory for all clinical purposes. The new procedure requires for its performance no more time and trouble than the system heretofore employed in the Prudential Laboratory.

Part I

CURRENT METHODS

To get as complete information as possible concerning the current practices of different laboratories, a questionnaire was sent to 100 laboratories which were known to specialize more or less in uranalysis and with whom we have had occasion, from time to time, to compare reports. These laboratories are scattered all over North America and are headed by men who come from different schools. The questionnaire deals with the various technical details involved in microscopic uranalysis, and I take this opportunity not only to acknowledge the 100 per cent responses, but also to express my obligations for the complete and careful answers, and especially to thank those who favored me with suggestions, opinions, experiences and, in a few cases, personal demonstrations, thus making the present effort practically a cooperative one.

As the problem concerned only equipment and manipulative details, the questionnaire was framed to elicit such information with the utmost simplicity and clearness.

QUESTIONNAIRE

A. Microscope:

1. Make of microscope?
2. Tube length employed?
3. Achromatic or apochromatic system?
4. Maker's designation of eyepieces used?
5. Maker's designation of objectives used?
6. Combination of eyepiece and objective used for 1/6 field?

B. Sediment:

1. Quantity of urine centrifuged?
2. At what speed?
3. For how long?
4. Is all or only part of sediment examined?

5. Please give full details of your technic for sampling sediment and preparing slide.
6. Size of slide used?
7. Size of cover glass used?
8. Do you examine more than one slide? If so, how many?
9. How many fields do you cover with low power? With high power?
10. Have you experimented with any other methods? If so, please give details, etc.

It will be seen that the first Part A of the questionnaire covers equipment. It develops the fact that over 95 per cent of the laboratories work with the achromatic systems of standard American microscopes. In this connection, it is not amiss to note that it is particularly in the examination of unstained and colorless specimens, such as urinary sediments, that the advantages of better resolution and freedom from chromatic aberration offered by apochromatic systems more than compensate for their increased cost.

The laboratories use eyepieces running all the way from 2 to 15 power but substantially conform with one another in the employment of 16 and 4 mm. objectives for uranalysis. One or another of every combination of these lenses is in use by the laboratories for counting the objects in urinary sediments, which, of course, causes discrepancies because the area of a microscopic field varies with a particular lens combination, even with a particular make, although 16 mm. objectives as a rule cover an area eighteen times greater than 4 mm. objectives of the same make.

For generations the textbooks on clinical pathology have recommended 4 mm. objectives for urinary microscopy, and it is undoubtedly true that the 4 mm. or even a higher power objective is essential for satisfactory resolving structural and cytologic details, and that every microscope used for examining urinary sediments should carry a high power objective on its nosepiece. Indispensable as it is, however, for seeing fine details, the 4 mm. or higher power objective is not only unnecessary but even disadvantageous and inexpedient for counting objects which can be easily identified with lower powers or less magnification. The higher the power of an objective the smaller is the field it covers, the shorter is its working distance, the less its penetration, the more finicky its focusing, and the greater its sensitiveness to thickness of cover glass and specimen. The greater penetrations afforded by 16 mm. objectives enable them to disclose objects lying in different planes which are much more likely to remain unseen and therefore uncounted when 4 mm. objectives are used. Those who feel that 16 mm. objectives do not give them sufficient magnification for counting would do well to try an 8 mm. objective. I have found them extremely well suited for counting the objects in urinary sediments because they give magnifications intermediate between 4 and 16 mm. objectives and are more comfortable to work with than 4 mm. objectives.

To sum up microscopic equipment, the questionnaires indicate such wide differences in practice between the different laboratories that I am led to suggest that better uniformity in reporting can be secured by an understanding to consider some definite area or some certain fixed volume as a standard unit for counting objects in urinary sediments. Then by some simple correction

factor, each individual or laboratory with the lens equipment they prefer can nevertheless put their reports on the same footing with other laboratories. As an example, the Prudential Laboratory employs apochromatic systems and counts with 8 mm. objectives and 15 power compensating eyepieces. This combination covers an area between two and three times the area covered by the usual 10 power eyepiece and 4 mm. achromatic objectives, but our reports become comparable and harmonize with those who count with 10 power eyepieces and 4 mm. achromatic objectives by the simple process of dividing our counts by 2½. Microscope makers readily supply data concerning the areas covered by their lenses, and the simplest kind of calculation will enable any individual using any equipment he prefers to report his findings uniformly with others and thus eliminate those discrepancies which are due to differences in microscopic equipment.

The second Part B of the questionnaire covers the manipulative and other details included in examining and reporting counts of red blood, pus, and epithelial cells and casts, etc., in urinary sediments. In general, the answers to the questionnaire expose a very disconcerting diversity of laboratory practice. In fact, the answers indicate that it is doubtful if any two laboratories make microscopic examinations of urine in exactly the same way.

As the questionnaires were returned, they were reviewed and roughly classified as to general technical design with special reference to sensitiveness and quantitative accuracy. To illustrate: one laboratory examines the sediment of 120 c.c. of urine and another that of only 5 c.c. If both laboratories received the same urine and examined the entire sediment, as some do, one would report 100 pus cells while the other would report about 4, perhaps none at all, because the technic of one laboratory is 25 times more sensitive than that of the other. On the other hand, when 5 and 120 c.c. samples of the same urine were treated differently, following details actually given in the questionnaires, the 5 c.c. sample sometimes yielded more sensitive or positive results than the 120 c.c. sample, or 25 times more urine.

From this it is evident that the problem of defining a microscopic procedure capable of giving quantitative and therefore uniform results was only to be solved by checking, step by step, the various technical procedures delineated by the answers given in the questionnaires. In order to make our study as complete as possible, other technics found in the textbooks and literature such as those of Addis,[1] Ockerblad,[2] Heitzmann[3] and others were also investigated. For the sake of convenience and clarity and to guide our experiments, all of the manipulative details involved in urinary microscopy were listed as follows:

SAMPLING

Sampling the urine.
Sedimenting the urine.
Sampling the sediment (microscopic specimen).

MICROSCOPY

Preparing the specimen.
Examining the specimen.
Reporting the findings.

It cannot escape attention that each step on the list connotes the performance of several distinct technical details; also that it was obligatory to perform the same manipulations many times and in various ways in order to gain the experience and data essential for comparing with and checking against one another all of the different technics outlined in the literature and questionnaires comprised in our program. From this it will be understood that this paper would run to an inordinate length were the attempt made to set forth fully all of the protocols accumulated in the course of the work. It is therefore necessary to limit the presentation of data to forms of summary or illustration in commenting on particular technical details.

SAMPLINGS

Sampling the Urine

If it is intended to examine the urine immediately after voiding or if the design be to sediment by gravity, no mixing is required. In all other instances the whole amount of urine voided should be well mixed before a sample is taken for examination or mailing. As a pitfall of frequent error, the importance of proper sampling cannot be overemphasized, and is experimentally demonstrable by the thousands of percentage differences in results found when different parts of the same insufficiently mixed urine are examined. No attempt to quantitate can succeed if the sample selected for examination be not true or representative. To insure thorough mixing, gently turning the bottle upside down a few times has proved as satisfactory as any other method, but it is particularly noteworthy that violent agitation is not only unnecessary but positively harmful because it tends to injure casts and otherwise damage the fragile objects we intend to examine under the microscope. It is a good plan to clear the specimen as far as possible of interfering suspended matter like urates, etc., before taking samples.

According to the questionnaires, almost all laboratories sample 15 c.c., a few sample 10 c.c., and a few only 5 c.c. On the other hand, some laboratories sample 2 ounces, or twelve times more, and others 4 ounces, making a 25-fold variation in the sample of urine examined by different laboratories. On this point a long line of related experiments indicate that the volume of urine sampled is relatively unimportant and that no particular advantages accrue from sampling larger amounts than the usual 15 c.c. sampled by more than 85 per cent of the laboratories. It is the efficiency of sampling and subsequent technic of handling rather than the amount of the sample which determine the sensitiveness and quantitative accuracy of the final result.

Sedimenting the Urine

The questionnaires develop the fact that only one laboratory relies on gravity alone and that all of the others depend more or less upon the centrifuge for sedimenting urine. Some laboratories, however, allow the urine to stand for a time, sedimenting by gravity before centrifuging a sample selected from the bottom layers.

The controversy over the relative merits of sedimenting urine by gravity or centrifuge centers chiefly on statements made by some authorities in text-

books and discussions to the effect that rapid centrifugalization deforms and injures fragile objects like leucocytes and casts. The point is important but, unfortunately, does not lend itself to illustration by experimental proof. I have, however, on numerous occasions sedimented by gravity and by centrifuge (2 to 3000 r.p.m.) urines containing not only blood, pus, and epithelial cells but also urines containing more fragile objects like waxy and fatty casts and hyaline and fatty detritus and then compared the sediments under the microscope. What I have seen convinces me that centrifugal speed within such limits as I have used it does not injure nor deform any of these objects unless centrifugalization be unduly prolonged. In this case violence is done by the packing of the sediment which takes place, and insult is added to injury in the process of extricating some of the sediment for examination. Other injurious forces come into play during deceleration of the centrifuge and in pouring off the supernatant urine or part of the sediment, because during deceleration and pouring eddies form which produce torques that exert unequal forces and thus tend to injure delicate objects by twisting them. On the other hand, centrifugal force is constant, steady and evenly distributed, and therefore much less likely to injure delicate objects. By avoiding unnecessarily prolonged centrifuging in order to prevent packing the sediment and by decelerating smoothly, a better conditioned sediment can actually be had with the centrifuge than by gravity.

The only other advantage which preliminary sedimentation by gravity might be conjectured to offer is increased sensitiveness, and on this point the methods given in the questionnaires were checked experimentally. Thus, the most sensitive technic given in the questionnaires was described as follows: "We plan to have a 4 ounce specimen. This is allowed to stand for at least one hour and the top three-fourths or four-fifths is poured off. Fifteen c.c. of the remainder is centrifuged for three minutes at 1050 r.p.m." The efficiency of this procedure was checked by carefully performing every detail of the technic on a specimen of normal urine to which was added fresh blood from a finger prick. Blood counts showed that the specimen contained 244,800,000 red cells. After standing an hour sedimenting by gravity, the upper three-fourths, or 90 c.c., were poured off and 15 c.c., or half of the remainder, were centrifuged three minutes at 1100 r.p.m. The red cells in the sediment were then counted and only 17,612,000 found, indicating a recovery of only about 60 per cent of the actual number of red cells in the 15 c.c. sample (hemolysis controlled). As a more sensitive criterion the red cells in the urine overlying the sediment were also counted, and both counts were found to agree within experimental error. A number of correlative experiments also indicate that the effect of an hour's preliminary sedimentation by gravity is negligible in the absence of efficient centrifugalization.

The questionnaires also reveal that some laboratories underdo and other laboratories overdo centrifuging. Thus one laboratory centrifuges only one minute while several centrifuge fifteen minutes, and one or more laboratories centrifuge for each interval between these. Similarly, the different labora-

tories centrifuge urine at different speeds ranging from 500 to 3000 r.p.m. with various r.p.ms. in between the extremes practiced by one or more of the laboratories.

No good reason for such wide variations in the performance of a routine technical detail are apparent, because, after all, the shaping of an adequate or efficient sedimenting technic is a simple matter of mathematics and mechanics which easily lends itself to experimental proof. Fourteen years ago I centrifuged urines containing casts and red and white blood cells at different r.p.ms. for different intervals of time in order to determine the time and speed factors necessary to bring 90 per cent or more of the microscopic objects in urine down into the sediment, and as a result the Prudential Laboratory has since then centrifuged urines for four and a half minutes at 2000 r.p.m.[4] This technic employs the usual 15 c.c. centrifuge tube and has proved efficient, and does very little, if any, injury to the microscopic objects. These early experiments have been rechecked during the course of the present study, and Tables

TABLE I

NORMAL URINE + RED BLOOD CELLS		CENTRIFUGED AT 2000 R.P.M.	NO. CELLS FOUND IN SEDIMENT	NO. CELLS FOUND IN SUPERNATANT URINE
VOL. C.C.	RED CELLS	MINUTES		
5	19,600,000	1	CF*	200
		2	CF	80
		3	CF	44
		4	CF	36
5	196,000	1	84	16
		2	84	0
		3	96	0
		4	108	0
10	47,800,000	1	14,240	432
		2	CF	248
		3	CF	56
		4	CF	40
10	478,000	1	432	108
		2	520	68
		3	560	8
		4	960	0
15	72,900,000	1	24,400	13,600
		2	46,400	2,400
		3	CF	1,600
		4	CF	410
15	729,000	1	260	410
		2	308	100
		3	350	80
		4	510	56

*Cover the field.

I and II give the results of centrifuging and counting experiments. Two different types of laboratory centrifuges with speeds controlled by tachometers were used. Counts were made with standard hemacytometers and Euscope.

The experimental results were confirmed with specimens from cases of hematuria and pyuria.

TABLE II

MINUTES AT 2000 R.P.M.	CELLS PER C.C. REMAINING IN SUPERNATANT URINE	PERCENTAGE OF REMOVAL
	Hematuria	
0	1,360,000	–
2	160,000	89.1
3	20,000	98.5
4	–	100.0
	Pyuria	
0	490,000	–
2	60,000	87.8
3	45,000	89.8
4	10,000	98.0
5	–	100.0

From these data it is evident that 15 c.c. urine samples in the usual type of centrifuge tube employed by practically all laboratories are efficiently and adequately sedimented by centrifuging four minutes at 2000 r.p.m. (As a practical matter, four and one-half minutes includes bringing the centrifuge to speed.) If greater or less speed than 2000 r.p.m. be preferred, the correct time factor for that speed should be determined. It should bring 98 per cent or more of the microscopic objects down into the sediment without being prolonged enough to pack the sediment.

All of the experiments shown in the tables were carried out with standard laboratory 15 c.c. centrifuge tubes, and the marked differences in the results obtained by centrifuging different quantities of urine, i.e., 5, 10 and 15 c.c., will not have escaped attention. At first sight it is not surprising to find that smaller quantities sediment quicker than larger quantities, but it would be a mistake to attribute the differences merely to volume of sample, because in the standard 15 c.c. centrifuge tube the samples stand at different heights or vary in length according to their quantity. Thus a 5 c.c. sample is 53 mm., a 10 c.c. sample is 80 mm., and a 15 c.c. sample 110 mm. long.

To determine the effect of length of sample on centrifuging, three tubes of different bore and shape were selected. As a matter of convenience these were standard types of centrifuge tubes designed to hold different quantities of sample, i.e., a 50 c.c. tube having a bore at the mouth with a diameter of 27 mm., a 30 c.c. tube with a diameter of 26 mm. at its mouth, and a 15 c.c. tube having a diameter of 14 mm. Into each of the three tubes, 15 c.c. of the same urine containing red blood cells was poured, and the lengths of the standing samples measured. In the 50 c.c. tube the urine sample measured 31 mm.; in the 30 c.c. tube 62 mm.; and in the 15 c.c. tube 95 mm. The samples were centrifuged different periods of time at 2000 r.p.m. and counts then made of the cells recovered from the supernatant urine which was centrifuged ten minutes at 3000 r.p.m.

The data strongly suggest a picture of sedimentation showing the microscopic objects lying in layers piled on top of one another, and the time factor for efficient centrifuging is, therefore, the time it takes to bring the topmost layers of microscopic objects in the sample down into the sediment. Correla-

tive experiments indicate that increasing the time factor by centrifuging at low speeds tends to irregularities in results and to injury of the microscopic objects by packing and other forces.

The data also point to the possibility of improving sedimentation technics by employing shorter centrifuge tubes.

TABLE III

EFFECT OF LENGTH OF SAMPLES ON CENTRIFUGALIZATION

MINUTES CENTRIFUGED AT 2000 R.P.M.	LENGTH OF SAMPLE	CELLS IN SUPERNATANT URINE
	31 mm.	65,000
1	62 mm.	120,000
	95 mm.	430,000
	31 mm.	0
2	62 mm.	90,000
	95 mm.	130,000
	31 mm.	0
3	62 mm.	20,000
	95 mm.	56,000
	31 mm.	0
4	62 mm.	0
	95 mm.	20,000

Sampling the Sediment (Microscopic Specimen)

After sedimenting urine, the next step is to get a good and true sample of the sediment for microscopy. The literature and questionnaires offer many and various ways for performing this step, and as a criterion for checking their accuracy I adopted the reproducibility of results obtained by repeating the same procedure on portions of the same urine.

Sediment must first be extricated from the supernatant urine. Many simply pour this off while others depend upon different methods of pipetting. When checked experimentally, none of the methods gave reproducible results. In fact, none of them could be managed so as to avoid diluting the sediment with urine, which always drips back from the sides of the tubes or enters into the pipettes. Such unavoidable dilutions of sediment represent an uncontrollable and inconstant factor which spoils every other attempt at accuracy, and from specimens which are poor in mucus or binding material for the sediment, such as low gravity urines, etc., casts are lost as easily by one method as by another. Furthermore, the amount of mucoid binding material almost always determines the volume of sediment obtained by centrifuging. It varies greatly in both normal and pathologic urines and is, therefore, a serious factor in destroying accuracy. The specimens from Messrs. X and Y illustrate this effect because both contain the same number of casts. X's urine is poor in binding material and yields only 0.1 c.c. of sediment, while Y's urine is richer in mucoid material and when similarly centrifuged yields 0.5 c.c., or 5 times more sediment. When both sediments are examined under the microscope and casts are counted with equal efficiency, the reports must show that X's urine contains 5 times as many casts as Y's. Such differences in sediment

concentration, of course, account for errors in reporting because specimens like X's are reported as having persistent casts while those like Y's are reported negative when both urines actually contain exactly the same number of casts.* The mucoid material present in urines has thus the same disastrous effects on accuracy as the unavoidable dilution which occurs in extricating sediment from supernatant urine.

These serious sources of error can be minimized, in fact avoided by scratch-marking the centrifuge tubes at some definite level so as to define some arbitrary volume of sediment or sediment plus urine which can always be taken as a constant sediment volume. To determine a practicable volume for such a constant, 100 consecutive urines having a specific gravity of 1020 or higher were centrifuged for four and a half minutes at 2000 r.p.m. with the results shown in Table IV.

TABLE IV

NO. OF SPECIMENS	VOLUME OF SEDIMENT
15	Less than 0.1 c.c.
19	" " 0.2 c.c.
32	" " 0.3 c.c.
31	" " 0.4 c.c.
2	" " 0.5 c.c.
1	" " 0.6 c.c.

Evidently 0.5 c.c. sediment or sediment plus urine is a practicable volume constant for 15 c.c. urine samples. This represents a concentration factor of 30. For the rare specimens which yield more than 0.5 c.c. of sediment it is easy to take 1 c.c. sediment or sediment plus urine and maintain the same concentration relation by multiplying results by two. Such sediments give us always the same concentration factor, i.e., they represent a 30-fold concentration of urine.

The next manipulation in order concerns getting a satisfactory sample of sediment on the microscope slide. Here again the questionnaires and literature indicate a rich variety of means by which a routine technical step is performed in different laboratories. For convenience of comment they may grossly be divided into those who attempt to examine all of the sediment and the majority, who are content to examine a sample of the sediment. Those who try to examine all of the sediment transfer it to a slide in several different ways. They introduce a pipette through the supernatant urine or decant the supernatant urine and then pour or transfer the sediment with a pipette. When these methods were tried out exactly as described and with different kinds of pipettes managed in different ways, it was found impossible to get all of the sediment on the slide by any of them. The texture of the sediment, its viscosity and surface tension, to say nothing of numerous extraneous factors like dilution and loss on glass surfaces and wetting qualities, prevent this operation being carried out with a reasonable degree of uniformity or accuracy by any of the methods. Other elements of inaccuracy also present themselves in making microscopic preparations of total sediments.

Those who are content to sample sediments employ different kinds of pipettes in various ways for the purpose and many specifically mention capil-

lary pipettes. What particular advantages capillarity or capillary pipettes afford in sampling urine sediments I have been unable to fathom. After seeing some of the pipettes in use and reading the descriptions of the ways they are used, I am unable to come to any other conclusion than that on this point there is very much misunderstanding and confusion.

The word capillary comes from the Latin capillus meaning hair, and glass blowers tell me that to them a capillary pipette means one whose walls are very thick in proportion to the bore. Such pipettes are less sensitive to temperature changes and therefore better for volumetric measurements. From what I can learn, the capillary pipettes alluded to in the questionnaires are not of this type. Glass tubes with hairlike bores are sometimes called capillary pipettes because in a restricted way mobile liquids will rise in them by capillary attraction. The viscosity of urinary sediments, however, nullifies whatever capillary attraction they may possess for more mobile liquids, and they are positively disadvantageous for handling urinary sediments because the narrowness of their bores, particularly at the tips, tends to break up casts and organized detritus which may be in the sediment. They also tend to selectivity in sampling which is even more objectionable. Therefore, 2 mm. may well be regarded as minimum and 3 mm. even better for the bore of pipettes designed for handling urinary sediments.

If a pipette or any other kind of a hollow tube, open at the ends, be introduced into a vessel containing a mobile liquid, the liquid rises in the tube to a level even with or perhaps a little higher than the surrounding liquid. The liquid in the tube gets to the level of the surrounding liquid by hydrostatic balance, and only that which rises above the level of the surrounding liquid is accounted for by capillarity, a weak and variable force that is hardly ever brought into play in handling urinary sediments. The technics which get up the sediment by introducing a pipette with upper end closed through the supernatant urine until the tip is in contact with the sediment depend upon the rush of urine into the pipette when the pressure of the finger is released to carry along the sediment with it, and not at all upon capillary forces.

It is thus evident that capillary pipettes are useless and disadvantageous for sampling urinary sediments. Some textbooks and questionnaires give no reasons but specifically warn against suction. Nothing has ever occurred in my experience to indicate that suction is any more disadvantageous than other forces brought into play by the different technics. In fact, suction has possibilities of substantial usefulness. The real point seems to be that only mild forces should be used in handling urinary sediments and that all forms of violence must be avoided.

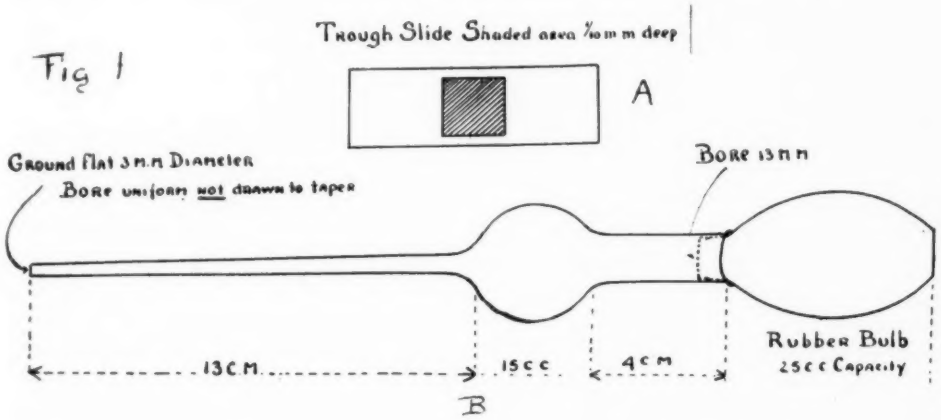
The technics of the laboratories that regularly examine total sediments are evidently designed to eliminate uncertainties of sampling. After practicing the manipulations and taking every precaution to insure uniformity, these technics were repeated on samples of the same urines and none of them gave reproducible results.

The laboratories that sample sediments take different amounts for examination. Some stress the importance of mixing sediments before taking sam-

ples; others take samples "from the top, middle and bottom of the sediment with a pipette" and mix them on a slide. When checked experimentally, it was found that mixing the sediment before sampling gives better reproducibility than attempts to sample the sediment by layers and then mix.

Thus, urine containing blood cells was centrifuged and samples selected both by picking "representative samples from the top, middle, and bottom of the sediment" and by mixing the whole sediment before selecting the sample. The average deviation between counts of eleven samples selected by the layer technic was 15.5 per cent, while the samples selected from the mixed whole sediment deviated only 5.3 per cent.

The only detail concerned with sampling sediments which remains is the method of mixing. Some stir the sediments with a rod, some tap, some rotate, and some shake the tube, others blow the sediment in and out of a pipette or jiggle the pipette up and down in the sediment. When checked against one



another, no great differences are apparent, but the results distinctly favor the last two methods, and this finding is corroborated by the whirling currents set up by the other methods which can be clearly seen to cause the microscopic objects to gravitate away from the periphery and form a thin revolving column in the center of the tube.

From the sediment sampling experiments it is clear that the volume of sediment should always be the same and that the sediment or sediment plus urine contained in the suggested 0.5 c.c. sediment volume constant should be thoroughly mixed before taking the sample for microscopy. To transfer the sample from centrifuge tube to slide, the suction pipette (Fig. 1) has been found satisfactory and convenient. Its length conforms with the usual 15 c.c. centrifuge tube. It is made of 3 mm. glass tubing plane ground at the tip and dilated near the upper end sufficiently to hold 15 c.c. With the rubber bulb compressed the tip is introduced into the centrifuge tube until near the 0.5 c.c. mark; the supernatant urine is drawn into the pipette by releasing, and then ejected by compressing the rubber bulb. The same pipette is then used to mix the sediment and transfer a sample of it to the slide.

MICROSCOPY

Preparing the Specimen

According to the questionnaires, 80 of the 100 laboratories employ 3 by 1 inch and 20, larger slides in order to accommodate either the entire sediment of 15 c.c. or multiple specimens. The questionnaires also reveal that 64 laboratories always use cover glasses, that 24 are indifferent about the matter and that 12 laboratories never use cover glasses for urine sediments. When the different technics outlined in the questionnaires were checked experimentally, it was found that the procedure with cover glasses always gave better results than the same procedure without them, and it is, of course, true that every optical and many other considerations decidedly favor the use of cover glasses.

Almost every possible way of preparing a microscopic specimen is included in the methods delineated in the questionnaires, and to try them out in detail required more time and care than all the rest of the work taken together. It is, nevertheless, again necessary to record failure because no matter how painstakingly performed, or how combined, none of the technics gave results which can be fairly called either quantitative, accurate, or reproducible.

In the course of the experiments it became apparent that irregularities and inconsistencies were, to some extent at least, due to certain characteristics of sediments which cannot be controlled, such as wetting quality, viscosity, and surface tension. It also became obvious that all microscope counting methods are hopeless unless made under conditions which insure an even volume and thickness of specimen and an even distribution of the microscopic objects therein.

To secure such conditions, Addis, Ockerblad and others resorted to the blood counter, and it was employed extensively throughout this work on both urines and sediments for experimental as well as control purposes. In fact, it was used so very often that an unusually rich experience in counting with it was gained from which were derived certain very definite impressions. Thus, although sediments were introduced into the counting cell in every conceivable way, from the standpoint of reproducibility our counts continued irregular and inconsistent, and this experience was taken to indicate that the blood counter is not as well adapted to sediments as it is to more mobile materials like urine and diluted blood. Other features of the blood counter which I think make it less suitable for uranalysis are the rulings which are confusing and account for much of its cost, and its very inadequate surface area with consequent limitation of content.

For counting the microscopic objects suspended in uncentrifuged urine, the blood counter is undoubtedly the best means at our command and must be considered standard even though filling and refilling the cell with different samples of the same urine is so tedious and time consuming as to render its employment in routine uranalysis hopelessly impracticable. In this work, for instance, the minimum requirement has been to count 400 squares on each of four different samples of the same well mixed urine even when the objects in 1 c.c. of the specimen ran into millions, and in proportion as the objects in the

specimen grow scarce, it is, of course, well known that more and more counts are necessary not only for accuracy but even to avoid missing their presence altogether.

With the idea of trying to dodge its disadvantages and at the same time to utilize the advantages of the blood counter, an experimental cell was constructed which is nothing more or less than a 3 by 1 inch slide having in its middle a 0.1 mm. deep trough 400 mm. square. Such a trough holds 400 times more sediment than the blood counting cell and is easily and quickly filled, in fact, quicker than by many current methods of preparing specimens because all one has to do is to transfer the sediment to the trough with a pipette and press the 0.5 mm. thick cover glass down around the rim of the trough. When made in this way, microscopic preparations always have the same volume and thickness and uniform distribution of microscopic objects throughout the specimen. Table V shows the correspondence of counts made on a series of urines with the blood counter (average of four or more samples) and sediments from the same urines in trough cells (average of 10 fields). The counts were made in the course of the usual routine of the Prudential Laboratory by six different technicians.

TABLE V

COUNTS OF BLOOD AND PUS CELLS IN URINE BY HEMACYTOMETER AND IN SEDIMENTS OF SAME URINE BY NEW METHOD

	COUNT PER C.C. URINE				COUNT PER C.C. URINE		
SPECIMEN	HEMACYTOMETER	TROUGH	DIFFERENCE PER CENT	SPECIMEN	HEMACYTOMETER	TROUGH	DIFFERENCE PER CENT
pus	82,000	74,000	9.6	blood	62,500	59,900	4.2
blood	50,000	49,000	2.0	blood	52,000	48,000	7.7
pus	95,000	90,000	5.6	pus	37,500	39,200	4.6
pus	160,000	152,000	5.0	blood	12,000	12,000	0.0
blood	27,470	26,700	2.7	pus	37,000	36,100	2.4
pus	27,470	26,700	2.7	pus	42,400	46,200	9.0
pus	40,000	42,000	5.0	pus	14,300	14,000	2.1
blood	25,000	22,400	10.4	blood	7,000	7,000	0.0
pus	280,000	268,000	4.3	pus	145,000	136,400	5.6
blood	270,000	248,000	8.1	pus	50,000	52,600	5.2
blood	17,500	16,500	5.7	blood	115,000	112,000	2.6
pus	15,000	13,300	11.3	pus	15,000	15,400	2.7
blood	20,000	18,000	10.0	pus	20,000	20,200	1.0
blood	45,000	40,300	10.4	blood	25,000	24,100	3.6
blood	75,000	83,300	11.1	pus	17,000	17,100	0.6
pus	22,500	21,000	6.7	blood	12,500	12,200	2.4
pus	17,000	15,400	9.4	pus	10,000	9,800	2.0
pus	125,000	115,500	7.2	pus	17,500	18,200	3.9
pus	142,500	156,700	10.0	pus	450,000	407,000	10.0
blood	87,500	77,400	11.5	blood	255,000	241,500	5.3

Average hemacytometer—trough cell difference 5.6 per cent

Examining the Specimen

According to the questionnaires, one laboratory tries to examine the entire sediment with both low and high powers, but only rarely are attempts made to cover more fields with high than with low powers. Some cover the same selected fields with both high and low powers but the majority employ high powers only "rarely," "casually," "when needed," "for confirmation," etc. The number of fields regularly examined run all the way from "several" or five to more than a hundred.

In order to lose no unnecessary time on negative ones, experienced microscopists generally begin their examination of a sediment by carefully but rapidly searching through a number of low power fields. Some such combination as 16 mm. objective and 10 power eyepiece giving a magnification of 100 or more suffices for this purpose, and the greater areas covered by low power objectives enable the microscopist to avoid wasting time and tedium over specimens which contain nothing of interest. In his searching the experienced microscopist, if he sees anything which is not clearly and unquestionably distinguishable or recognizable, will resolve it by switching on the high power objective. The technician-microscopist if he has any doubt at all about the identity or significance of an object thus resolved, is duty bound to show it to the director of the laboratory, who, it is assumed, is a pathologist. Technicians should be continually reminded that the principal virtues of a microscopist are keenness and discrimination in spotting unusual and unfamiliar objects and honesty and accuracy in counting objects which have to be counted when they are present in the specimens. Counting objects like casts and pus cells in unstained specimens is tedious work, and the use of higher magnifications than are necessary for comfortably resolving and clearly distinguishing the objects which have to be counted tends to diminish accuracy because of the optical restrictions imposed by higher powers. Similarly operative as another deterrent to accuracy is the thickness of specimen which makes it necessary to focus on objects lying in different planes.

Reporting the Findings

It is now customary to report microscopic findings by numbers counted or by words like rare, many, few, etc. Such words are indefinitely intriguing to many, and it may be possible to conventionalize them so as to convey more or less definite information. Until such conventions are well established, however, very different meanings attach to such words not only among those who report but also by those who interpret them.

On the other hand, reports which are made in terms of actual numbers tend to emphasize lack of uniformity and discrepancies, because numbers carry more concrete meanings than words. Also, circumstances such as those outlined in the paragraphs on microscope equipment, sampling, sedimentation, etc., give such reports false airs of accuracy which lead to a fool's paradise of misinformation and confusion with consequent irritations and embarrassments.

The remedy for the situation is a change in our habits of reporting. The plan of reporting the number of microscopic objects per unit of urine volume commends itself as being in accord with the nature of urine and having other advantages. It is now customary to report the cellular elements in urine by some equivalent of number per microscope field and to report casts in terms equivalent to the number per slide or total sediment. On the plan suggested all microscopic objects would be reported in the very same terms as so many per cubic millimeter or per c.c. or per 15 c.c. or per liter of urine, as may be preferred. At first sight it appears logical and appropriate to report urine

counts in the same terms as blood counts, but even when clinically significant, casts are apt to run less than 1 cast per cubic millimeter, and it would, therefore, be necessary to report them often in terms of a fraction of a cast per cubic millimeter, which is, of course, undesirable. On the other hand, writing more digits as the higher units of volume require is also undesirable. Therefore, the content of 1 c.c. of urine looks like the best compromise for a satisfactory unit of volume. Table VI has been compiled and calculated to show how simply counts of average sediment fields are convertible into counts per volume of uncentrifuged urine. Thus 20 cells in a high power sediment field equal 62,000 pus cells per c.c. of uncentrifuged urine and one cast in every tenth low power sediment field equals 17 in 1 c.c. of the original urine.

TABLE VI

TABLE OF FACTORS FOR CONVERTING MICROSCOPE COUNTS INTO EQUIVALENT NUMBERS PER UNIT OF URINE VOLUME

	ACHROMATIC			APOCHROMATIC
	10 OCULAR 4 OBJECTIVE	10 OCULAR 8 OBJECTIVE	10 OCULAR 16 OBJECTIVE	15 OCULAR 8 OBJECTIVE
Field				
Diameter	0.37	0.78	1.59	0.55
Surface area	0.108	0.478	1.986	0.238
Volume when depth is 0.1 mm.	0.0108	0.0478	0.1986	0.0238
Numbers per unit volume when count is 1 per field				
Per cubic millimeter	93	21	5	42
Per cubic centimeter	93,000	21,000	5,000	42,000
Per 15 c.c.	1,389,000	313,000	75,000	630,000
Per 15 c.c. concentrated 30 times (0.5 c.c. sediment)	46,500	10,500	2,500	21,000
Factor for converting count per sediment field to number in 1 c.c. urine	3,100	700	170	1,400

Reports of microscopic examinations of urine so often carry the word negative that it deserves to have a specific meaning assigned to it. The answers to the questionnaire suggest 20 low power fields as being generally acceptable. If such a convention were established, it would be possible to define a negative specimen as 4 c.mm. of urine which contained no casts and not enough cellular elements to make it worth while counting them. As a corollary, the word negative used in connection with a particular kind of urinary object would mean that there were not enough of them to be of interest in 4 c.mm. of urine.

Part II

The information developed in Part I enables the formulation of a practicable system of quantitative urinary microscopy. To facilitate its quick and easy performance the centrifuge tubes are scratch-marked at levels indicating 0.5 and 15 c.c. and two pieces of glassware (see Fig. 1), a syringe pipette, and a trough slide, have been designed. The pipette conveniently extricates,

mixes, and transfers the sediment; the trough slide facilitates counting by insuring a constant volume and thinness of specimen. Fewer fields need be counted than in the usual varied and uneven preparations. If one counts 4 fields, for example, and finds the distribution in the trough as even as it should be, the result multiplied by 5 gives the content of 20 fields.*

To apply the method:

1. Pour the well mixed urine into the centrifuge tube up to the 15 c.c. mark, and centrifuge four and one-half minutes at 2000 r.p.m. or the equivalent.
2. Withdraw the upper 14.5 c.c. of urine and mix the remaining 0.5 c.c. of sediment or sediment plus urine with the pipette (jiggle up and down).
3. With the pipette transfer the sediment to the trough and press the 0.5 mm. thick cover glass down around the rim.
4. Examine the slide under the microscope, and multiply or divide the appropriate factor (Table VI) by the number of objects found in an average field, and report the results as so many blood or other cells or casts in 1 c.c. of urine.

This system of urinary microscopy offers the following advantages:

1. It is quicker and easier to perform than current methods.
2. It is sensitive enough for every conceivable purpose.
3. It enables reporting all microscopic objects in the same terms.
4. It makes uniformity of reporting by different laboratories possible.
5. It is much more accurate than current methods.
6. It is a quantitative method, i.e., experimental error around 10 per cent.

In conclusion, I desire to express my thanks and indebtedness to Miss Gertrude Casey and Mr. John Huizer for efficient assistance and to Drs. P. V. Wells and Anton R. Rose for valuable suggestions and help.

Postscript

Since the foregoing paper was written a microscopic slide designed for counting mould cells was brought to my attention by the Bausch & Lomb Optical Company. In its original form it is impractical because unnecessarily cumbersome, complicated and thick. I therefore suggested a simpler and lighter construction which enables the use of high-power objectives and is very practical for urinalysis. The improved mould slide gives exactly the same results as the trough slide but is preferable because easier to make and also because cover glass fit is rendered less messy by a gutter surrounding the specimen which disposes of superfluities.

I have taken further advantage of this feature to secure extremely satisfactory slides which hold eight separate sediments under one cover glass. Notwithstanding greater cost such slides are proving actually more economical in routine use than the usual types of microscope slides, and besides other advantages their polished optical glass construction markedly improves the microscopic appearances of colorless objects like hyaline casts.

The technics have been further simplified by automatically measuring the constant sediment volume instead of pipetting off the supernatant urine,

*This statement must not be taken as contradicting the well-known principles that accuracy is greater in proportion to the number of fields counted.

which now drains out through a small hole drilled through the 0.5 c.c. mark of the centrifuge tube. Before centrifuging cover the hole with a section of rubber tubing and pour an inch or so of water in the cup which holds the centrifuge tube. After centrifuging, remove the rubber (with a small wire hook) and blow gently (breath or bulb) into the tube to overcome slight surface tension effects.

It may also be of interest to note that the examination of some 25,000 specimens by the new method shows very definitely that the correct results obtained greatly increase the clinical value of urinary microscopy.

SUMMARY

Part I

With a view to improving accuracy and uniformity of reporting, current methods of urinary microscopy were checked experimentally and the information developed recorded.

Part II

A simple and rapid quantitative method of microscopic uranalysis is described.

REFERENCES

1. Addis, Thomas: Determination of Extent and Nature of Renal Lesion in Bright's Disease, Calif. State J. Med. **20**: 90-93, 1922.
2. Ockerblad, Nelse F.: Accurate Method of Estimating Pus and Blood Cells in Urine, J. Urol. **19**: 525-526, 1928.
3. Heitzmann, Louis: Urinary Analysis and Diagnosis by Microscopical and Chemical Examination, 1928, Fifth rev. ed., Wood.
4. Exton, Wm. G.: The Relation of Urine to Life Insurance. American Life Convention, Medical Section 117-144, 1921.

DISCUSSION

Dr. Frederic E. Sondern.—Dr. Exton's article is most instructive, and the use of quantitative microscopic examinations may give us information we do not obtain from our present methods of estimating the amounts of the different ingredients of the urine sediment. The Rockefeller Institute Hospital of New York has been making its microscopic urine reports in the manner suggested by Dr. Exton and I am free to confess that with no experience in this regard, they have been difficult to interpret. It will take personal experience to coordinate the new enumeration with the old.

Dr. E. R. Mugrage.—We note that centrifuging always varies with different instruments, and the effect of gravity with one instrument will be many times that with another. We have learned with one instrument used in our laboratory that if we allow the centrifuge to run to 2000, there will be a certain distortion of urinary elements. Dr. Exton spoke of phosphates, and I presume he meant also amorphous urates. We have been in the habit of removing those urates by warming the specimen, and removing the phosphates by the addition of a small amount of acetic acid, in order that we do not get a distortion of the sediment of value, and that they can also be seen more readily.

Dr. Francis B. Johnson.—This point is a very important one particularly with each individual hospital and in hospitals where only interns are employed and to see that some standard be taken by which we can control and compare results. It is interesting that in my own work for a great many years we have used some standards similar to these. We have always centrifuged our urine to a 1:30 concentration. In some cases we have smaller quantities of urine, particularly in children, and we make allowances for that. In figuring out how to classify our findings we considered them more in regard to pus. It has been

agreed upon that when there are very few leucocytes found to the high power field under a cover glass we consider that as not positive; so on as 1, 2, 3, and 4. For instance 3 to 10 cells, 1-plus, 10 to 20, 2-plus, 20 to 30, 3-plus, 30 to 40, 4-plus. In looking for casts we felt that using a cover glass made a field so thin that it took too long to find them. The drop was placed on a slide and spread about the same depth as with a cover glass. We use two drops, one with and one without a cover glass.

There has to be a definite method for comparison to be able to check up where a great many men are making these microscopic examinations.

Dr. William G. Exton (closing).—May I say that in this work there was so very much repetition of manipulative detail that it was easily the most tedious and troublesome I ever attempted. I am, therefore, gratified to hear what Dr. Johnson has said because he has made me feel that the trouble taken was worth while.

As laboratory men we need methods that will enable us to report examination results which agree with one another because we lose the confidence of layman and physician and expose ourselves to criticism when our reports disagree. The method I have outlined for you gives exact and truthful answers instead of the slippery conventionalities we have been hitherto getting. It puts urinary microscopy on a definite quantitative basis and should, therefore, increase the clinical value of uranalyses.

As to some of the suggestions which have been made, I am careful not to add acetic acid or other chemicals to specimens before examining these under the microscope. I prefer to dilute if necessary rather than treat them with reagents which may change the microscopic objects.

The point in centrifuging is to centrifuge long and fast enough to bring the microscopic objects down into the sediment and to stop at this point, because longer centrifuging brings into play packing and other forces which injure the delicate objects we are interested in seeing and counting. I am sorry I omitted reading the part of the paper which deals with injury to microscopic objects, but you will find that centrifugal force is even and steady and not likely to injury the microscopic objects. When they suffer injury it is usually due to packing of the sediment or to forces which are brought into play in extricating the sediment from the supernatant urine. Dr. Sondern is very much to the point about the matter of coordinating correct enumeration with our present system.

ACUTE DIFFUSE MYELITIS FOLLOWING INTRAVENOUS INJECTION OF NEOARSPHENAMINE*

By Ernest Scott, M.D., and Harry L. Reinhart, M.D., Columbus, Ohio

THE report of the salvarsan committee of the British Medical Research Council on the toxic effects following the employment of arsenobenzol preparations is the most exhaustive compilation of facts and observations upon this subject in the literature today; and it is indeed pertinent that their conclusions are headed by the statements that "no special arsenobenzol preparation can be regarded as more likely than others to produce ill effects. Errors in technic cannot account for more than a few serious accidents; fatalities have occurred even under the most careful control in large and completely equipped hospitals."

From considerable experience and observation with various arsenobenzol preparations, and their administration under both the best and the worst technic, we are of the opinion that there is a wide range of tolerance in most individuals, and that the nature of the preparation and the technic are of less importance in the fatalities which may follow the administration of arsenobenzol preparations than the tolerance of the patient. It is for this reason that it is always advisable to begin a course of salvarsan administration with minimal dosage and increase it gradually to the therapeutic dose, observing the patient most carefully meanwhile. Even then we are not assured that we will encounter no fatalities. On the other hand, physicians should not so readily assume that their technic or favorite preparation is omnipotent in the prevention of fatalities, since the incidence of fatalities is about one in each 5,000 to 10,000 injections, or according to the German statistics, as cited by Phelps,[1] one death for every 3,788 cases treated. From such statistics it is at once apparent that most physicians may administer salvarsan preparations throughout their lives without encountering a single fatality.

Continuing the summary of the British Medical Research Council[2] the following are listed as the most important ill effects which may end fatally:

(a) "Encephalitis hemorrhagica,

(b) "Acute yellow atrophy of the liver,

(c) "Exfoliative dermatitis and its complications."

It is further pointed out that encephalitis hemorrhagica is most frequently described in the German literature, and exfoliative dermatitis and its septic complications have accounted for most of the fatal accidents in the British and American literature.

To the above list of ill effects, which may follow the administration of salvarsan, and which not infrequently result in death, we would add another con-

*Read before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.

Department of Pathology, Ohio State University, Columbus, Ohio.

dition, which while rare nevertheless is definite in its clinical and pathologic aspects. This condition is characterized by a *diffuse acute degeneration of both the white and grey substance of the spinal cord, with marked destruction of the ganglion cells of the anterior horns.* Clinically, we have designated it acute myelitis; pathologically, it resembles the hemorrhagic encephalitis which follows salvarsan injection, and like the latter condition it has been reported more frequently in the German literature than in the British or American literature. In fact, after careful search through the British and American literature of the last ten years we have been unable to find a single case which clinically has followed the course of this condition, and which has been carefully checked by autopsy.

The following case illustrates the clinical course of a case of acute myelitis following the intravenous administration of neosalvarsan.

CASE REPORT

E. A., twenty-three years old, white, male, married, well developed and nourished, entered the office of a private physician and requested a blood test. He did not complain of any particular symptoms, nor did he give his reasons for desiring a blood test. There was neither a history of luetic infection, manifested by a chancre, generalized rash or sore throat, nor on physical examination was there any evidence of active or latent syphilis. The report of the blood Wassermann was strongly positive for syphilis. He was immediately given an intravenous injection of 0.9 gm. of neoarsphenamine, during and after which he manifested no signs or symptoms of any sensitivity to the drug. Five days later he was given a second intravenous injection of 0.9 gm. of neoarsphenamine, during the administration of which he manifested no unusual symptoms. Approximately eight hours later he complained of a cold and was administered some cold medicine. Two days later he felt some better, except for general weakness and numbness of the feet and ankles. The following day the weakness and numbness of the lower extremities had extended to the hips. Upon examination he showed a loss of sensation up to the hips and marked weakness of the lower extremities, which amounted to a paralysis. In consultation with two other physicians, opinions differed as to the nature of the process, one being inclined to believe that it was an acute syphilitic, transverse myelitis, while the other believed it to be an acute anterior poliomyelitis. Thus, there was clinical evidence of a process involving both the white and grey substance of the cord. The process advanced rapidly until eleven days after the last injection of neoarsphenamine when he entered a hospital; the following notations were made: There was a complete flaccid paralysis of the body and lower extremities, while the upper extremities exhibited a paresis and paresthesia of the hands. The biceps reflexes were barely perceptible, all others being abolished. There was evidently a paralysis of the intercostal muscles as the breathing was abdominal in type, attended by a diminution in size of the chest on inspiration and expansion on expiration. Loss of sensation extended up to the second rib anteriorly and second dorsal spine posteriorly. The bladder was distended and at the level of the umbilicus. The sensorium was fairly clear at times.

The hospital laboratory reports were as follows: The blood Wassermann was negative; the spinal fluid Wassermann was positive, 2 plus, and the cell count of the spinal fluid was 19. The blood count revealed 4,200,000 erythrocytes with a hemoglobin of 78 per cent; the total leucocyte count was 14,880 with a differential count of 80 per cent polymorphonuclears, 13 per cent lymphocytes and 7 per cent mononuclears. The urine showed a 2 plus albumin with many polymorphonuclear leucocytes and erythrocytes.

A summary of the clinical course revealed a young man without definite clinical evidence of syphilis, who, following the second injection of neoarsphenamine, developed a reaction apparently due to the neoarsphenamine, which indicated injury to the spinal cord, as manifested by an extensive flaccid

paralysis and complete loss of sensation below the cervical cord, and rapidly progressed to a fatal termination within two weeks. The clinical diagnosis was arsenical, or luetic endarteritis of the vessels of the cord.

The autopsy was held the day following death, the body having been embalmed in the meantime. The anatomic diagnosis at the autopsy was: (1) edema of lungs and slight hydrothorax; (2) nephrosis; (3) splenomegaly; (4) dilatation of urinary bladder; (5) bilateral dilatation of the pelvis of the kidneys; (6) left suppurative pyelitis; (7) edema of brain; (8) acute diffuse myelitis; and (9) multiple decubital ulcers of back and sacrum. Primary cause of death was acute, diffuse, toxic myelitis following intravenous injection of neosalvarsan; secondary cause was general sepsis.

Fig. 1.—Marchi stain. Diffuse degeneration of myelin sheaths of all tracts, and multiple hemorrhages scattered throughout the cord. Both white and grey substance is involved and there is no inflammatory reaction present.

Since the clinical symptoms indicated definite injury to the spinal cord, it was examined with much interest. The vertebrae and the spinal canal were normal. The dura presented no pathologic lesions, nor was there any gross evidence of hemorrhage or thrombosis of the vessels of the cord. The consistency of the cord was decidedly pathologic, being so soft that it was impossible to section it cleanly with a sharp knife without crushing it. This condition was present from the lumbar region to the upper cervical region where it gradually approached a normal consistency. The surface of the cut section was moderately hemorrhagic but without evidence of suppuration or tumor formation.

Microscopic examination of the cord revealed a moderate edema and vascular congestion of the pia. There was no evidence of exudation or local proliferation of cells in the pia. The dorsal roots presented a slight, sparse degeneration of the myelin sheaths. The white substance of the cord was

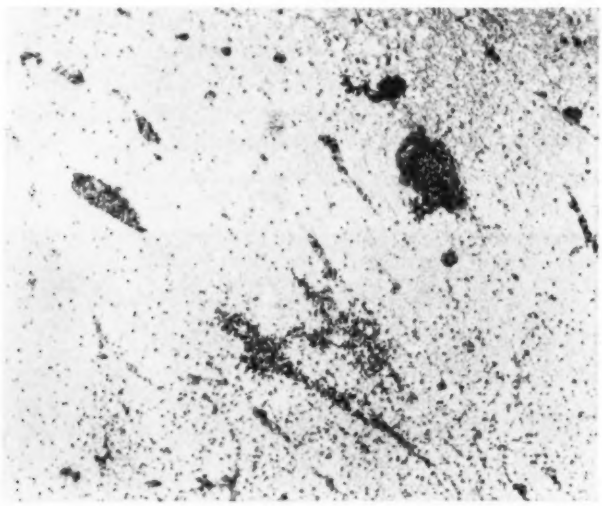


Fig. 2.—Columns of the cord showing vascular congestion, hemorrhage and hyaline degeneration of the walls of the blood vessels. There is considerable perivascular hemorrhage but no perivascular infiltration of leucocytes.

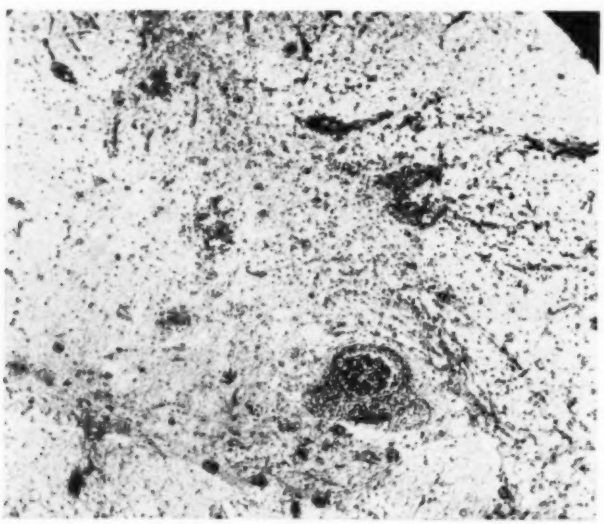


Fig. 3.—Anterior horn, showing complete degeneration of anterior horn ganglion cells, vascular congestion and hemorrhage.

markedly and diffusely degenerated. Practically every tract was extensively involved by this degeneration, which was all the more striking by the absence of any inflammatory reaction (Fig. 1). This degeneration extended from the lumbar to the cervical region, being most marked in the upper dorsal and

lower cervical region. The blood vessels were in various stages of degeneration, as manifested by edema of the cells of the walls, hyaline degeneration, thrombosis, congestion and rupture with perivascular hemorrhages (Fig. 2). The grey substance of the cord was likewise involved in the degeneration, the most striking pathology being the complete destruction of the ganglion cells of the anterior horns.

The picture in general conformed to the type of microscopic pathology seen in acute hemorrhagic encephalitis following the administration of salvarsan. Comparison of the cord pathology of this case with the brain pathology of the case of acute hemorrhagic encephalitis reported by Scott and Moore[3] revealed a striking similarity in type and distribution of the lesions. In many of the necropsies where a hemorrhagic encephalitis has been found following the administration of arsphenamine the cord has not been examined, which is unfortunate, since it does not reveal whether or not these two lesions are ever associated. In the present case there was no similar associated pathology in the brain.

A summary of the pathology of the cord reveals a diffuse myelomalacia, involving both white and grey substance, degenerative lesions of the blood vessels with perivascular hemorrhages, and complete destruction of all ganglion cells of the anterior horns (Fig. 3).

DISCUSSION

It is at once apparent that the most probable etiologic agents in this case are either syphilis or neoarsphenamine. The only evidence of syphilis is a positive blood Wassermann reaction in one laboratory test—a negative blood Wassermann in another following neoarsphenamine administration, and a positive spinal fluid Wassermann after the acute pathology had developed. There was no history or clinical evidence of syphilis. The gross and microscopic examinations of the various organs removed at autopsy presented no lesions which were even suggestive of syphilis. Therefore, we have at least eliminated syphilis pathologically and feel that the evidence for syphilis is minimal.

In classing this case as another one of those rare fatalities which occasionally follow the injection of arsenobenzol preparations, we note many points of similarity in the clinical course and pathology. Thus, for example, the reactions after intravenous arsenic preparations may be classed as immediate or delayed. In the case under discussion the reaction was not immediate but delayed. It occurred within twelve hours after the second injection of neoarsphenamine, which is the time of greatest incidence of delayed reactions. Furthermore, the delayed reactions are essentially cases of arsenic poisoning, and the pathology in this case is essentially that of arsenic poisoning.

Chiari[4] cites two cases of myelitis following poisoning with arsenous acid. He says that examination of the cords revealed degeneration of the ganglion cells in the anterior horns, with atrophy of the medullated network in the anterior horns and poverty of the fibers in the anterior columns, * * * * degeneration of Goll's columns in the cervical marrow and a circumscribed hemorrhage in the left anterior horn in the second lumbar segment. Chiari continues with a report of the cord pathology following the intravenous

administration of neosalvarsan as follows, severe disease of the seventh to ninth thoracic segments of the cord from which both ascending and descending degeneration passed out. This disease was not a true myelitis, such, for example, as that of epidemic poliomyelitis. It was rather a regressive change of the character of a necrosis which had affected both grey and white substance. There was marked destruction of ganglion cells, and the medullated network in the grey substance was entirely degenerated. Chiari is, therefore, of the opinion that his case of myelitis following the intravenous administration of neoarsphenamine was caused by the arsenic component of the drug. The similarity of the pathology of the two cases of arsenic poisoning, as well as the myelitis following salvarsan, agrees entirely with the pathology of our case.

Nonne[5] reports a fatal injury to the spinal cord after intraspinal salvarsan treatment, in which the predominant lesion was degeneration. He cites this case as the fourth case of severe incurable disease of the spinal cord after intraspinal use of salvarsan, and, while the other three cases were not autopsied, the symptoms were those of paralysis of the conus medullaris.

Mingazzini[6] also reports a hemorrhagic postsalvarsan myelitis, in the discussion of which he cites Nonne's case and states that it and his own case are two really classical cases of postsalvarsan myelitis in which the clinical symptomatology and the postmortem findings were in harmony. Our own case is in entire accordance with all cases cited, and conforms to the classical clinical course, and cord pathology.

ETIOLOGY

Many theories have been presented to explain the action of arsphenamine in the fatalities which have followed its administration. An excellent summary of these is given in the paper by Scott and Moore.[3] We should like to repeat this summary of the pathologic lesions as found in cases of "(1) experimental poisoning of animals, (2) the lesions found at autopsy in individuals who have died from poisoning with inorganic arsenic preparations, (3) those in which fatalities followed the administration of salvarsan."

"1. A destructive action on the endothelium resulting in congestion and hemorrhage in most of the organs of the body.

"2. The poor circulation resulting in edema of the brain and lungs in particular.

"3. A nephritis, usually of the tubular type, but under certain circumstances taking on also a glomerular type.

"4. The findings of infarcted and necrotic areas in the various viscera in some cases.

"5. Necrosis of the liver, and sometimes acute yellow atrophy."

Besides the characteristic pathologic picture of arsenic poisoning in the spinal cord our case presented evidence of arsenic poisoning in the destruction of the vascular endothelium and congestion, edema of the brain and lungs, and a nephrosis.

We are, therefore, of the opinion that the essential pathology is the result of the arsenic component of the neoarsphenamine. While it may be ques-

tioned that the results in this case would have been otherwise, still we believe that all administrations of salvarsan should begin with minimal dosage and slowly progress to the therapeutic dosage.

SUMMARY

1. A classical case of acute myelitis following the intravenous administration of neosalvarsan, in which both clinical and pathologic findings coincide, is presented.

2. The clinical course is that of an acute onset of extensive flaccid paralysis rapidly following the second intravenous administration of neoarsphenamine, and terminating in death in less than two weeks.

3. The pathologic cord findings are those of extensive degeneration of blood vessels, diffuse degeneration of white and grey substance of the cord, and complete destruction of ganglion cells of the anterior horn, without exudate phenomena or glial proliferation.

4. Such cases should be classed along with hemorrhagic encephalitis, as one of the potential causes of fatalities following the intravenous administration of arsphenamine.

REFERENCES

1. Phelps, J. R.: A Fatal Case of Acute Poisoning by Neoarsphenamine. Comment. U. S. Nav. M. Bull. **22**: 217, 1925.
2. Report of the Salvarsan Committee: II. Toxic Effects Following the Employment of Arsenobenzol Preparations, Medical Research Council, Special Report Series, No. 66, 1922.
3. Scott and Moore: Fatalities Following the Use of Arsphenamine, J. LAB. & CLIN. MED. **13**: 345, 1928.
4. Chiari, H.: Über eine nach Neosalvarsaninjektionen aufgetretene "Myelitis," Verhandl. d. deutsch. path. Gesellsch, 155, 1913.
5. Nonne, M.: Letale Rückenmarks-schädigung durch intra-spinale Salvarsanbehandlung, Deutsche Ztschr. f. Nervenh. **94**: 158, 1926.
6. Mingazzini, G.: Klinischer und pathologisch-anatomischer Beitrag zum Studium der Myelitis haemorrhagica postsalvarsanica, Deutsche. Ztschr. f. Nervenh. **104**: 1, 1928.

TRANSACTIONS

Minutes Eighth Annual Convention American Society of Clinical Pathologists Portland, Oregon, July 5, 6, and 8, 1929

The proceedings were held in the Portland Hotel, Portland, Oregon, July 5, 6, and 8, 1929.

The meeting was called to order Friday, July 5, 1929, at 9 A.M. by the President-Elect, Dr. J. H. Black, Dallas, Texas. Dr. H. H. Foskett, Portland, Oregon, Chairman of the Local Committee on Arrangements, made several announcements regarding entertainment planned for the Members. The proposed amendments to the Constitution and By-Laws were introduced and a motion was made and carried that they be brought up at the proper time Monday.

There being no further business at this time the following program was presented at the regular scientific session of the Society.

"The Effect of the Presence of Bile on the Agglutination Reaction." By Ruth Gilbert, M.D., and Marion B. Coleman, B.S., Albany, New York. Read by Dr. V. W. Bergstrom. No discussion.

"Recent Developments in Tularemia (Francis' Disease): With a Report of Eleven Additional Cases." By Walter M. Simpson, M.D., Dayton, Ohio. Discussion by Dr. J. C. Geiger, San Francisco; Dr. W. T. Cummins, San Francisco; Dr. C. W. Bonynge, Los Angeles; Dr. Warren T. Vaughan, Richmond, Virginia; Dr. Robert F. E. Stier, Spokane, Washington; Dr. Roy W. Hammack, Los Angeles; Dr. Frederic E. Sondern, New York City; and Dr. Walter M. Simpson, Dayton, Ohio.

"The Routine Use of Photoelectric Hemoglobinometer." By A. H. Sanford, M.D., and Charles Sheard, Ph.D., Rochester, Minnesota. Discussion by Dr. Walter M. Simpson, Dayton, Ohio, and Dr. A. H. Sanford, Rochester, Minnesota.

"Acute Diffuse Myelitis Following Intravenous Injection of Neoarsphenamine." By Ernest Scott, M.D., and H. L. Reinhart, M.D., Columbus, Ohio. Read by Dr. C. H. Fanlove, Portland, Oregon. No discussion.

"Spontaneous Meningeal Hemorrhage." By Frederick H. Lamb, M.D., Davenport, Iowa. Discussion by Dr. A. S. Giordano, South Bend, Indiana, and Dr. Frederick H. Lamb.

Friday Afternoon, July 5, 1929, 2 p.m.

The meeting was called to order by the President, Dr. F. W. Hartman who appointed a Nominating Committee as follows: Dr. Rawson J. Pickard, Chairman, San Diego, California; Dr. Robert A. Keilty, Washington, D. C.; and Dr. Walter M. Simpson, Dayton, Ohio. To fill vacancies on the Research Committee President Hartman appointed the following Fellows: Dr. Frederic E. Sondern, New York City; Dr. W. T. Cummins, San Francisco; and Dr. C. W. Maynard, Pueblo, Colorado. The scientific program was continued.

"Undulant Fever in Man: A Clinical Analysis of Thirty-Three Cases." By A. S. Giordano, M.D., and R. L. Sensenich, M.D., South Bend, Indiana.

"The Etiology and Diagnosis of Undulant Fever in the United States." By Charles M. Carpenter, M.D., and Ruth Boak, Ph.D., Ithaca, N. Y. (By invitation.)

"Some Observations on the Agglutination of B. Abortus." By Frank B. Lynch, Jr., M.D., and Annette M. Callan, Philadelphia, Pa. Read by Dr. Ralph Mills, Rochester, Minn.

"Notes on the Bacteriology of the Brucella Group." By K. F. Meyer, M.D., and B. Eddie, San Francisco, California. Read by J. C. Geiger, M.D., San Francisco, California. (By invitation.)

Papers discussed by Dr. Walter M. Simpson, Dayton, Ohio; Dr. F. W. Hartman, Detroit; Dr. J. C. Geiger, San Francisco; Dr. C. W. Maynard, Pueblo, Colorado; Dr. R. L. Sensenich, South Bend, Indiana; Dr. A. V. Hardy, Iowa City, Iowa; Dr. Roy W. Hammack, Los Angeles, Dr. C. W. Bonynge, Los Angeles; Dr. D. Schuyler Pulford, Woodland, California; Dr. E. R. Mugrage, Denver; Dr. A. S. Giordano, South Bend, Indiana; and Dr. Charles M. Carpenter, Ithaca, N. Y.

Friday Evening, July 5, 1929, 7 p.m. Round Table Discussion

The meeting was called to order by President F. W. Hartman. The first subject for discussion was presented by Dr. Philip Hillkowitz, Denver, entitled, "Virtuosity in Clinical Pathology." Discussion by Dr. F. W. Hartman, Detroit; Dr. William G. Exton, Newark, N. J.; Dr. Frederick H. Lamb, Davenport, Iowa; Dr. Frederic E. Sondern, New York City; and Dr. Philip Hillkowitz.

"Problems." By W. G. Gamble, Jr., M.D., Chicago, Illinois. Discussion by Dr. William G. Exton, Newark; Dr. Frederic E. Sondern, New York; Dr. Charles R. Drake, Minneapolis; Dr. Warren T. Vaughan, Richmond, Va.; Dr. B. W. Rhamy, Fort Wayne, Indiana; Dr. Zera E. Bolin, San Francisco; Dr. Philip Hillkowitz, Denver; Dr. Francis B. Johnson, Charleston, S. C.; Dr. Roy W. Hammack, Los Angeles; Dr. C. W. Bonynge, Los Angeles; and Dr. W. G. Gamble, Chicago.

"The State Laboratory Problem." By Frederic E. Sondern, New York City. Discussion by Dr. B. W. Rhamy, Fort Wayne, and Dr. Frederic E. Sondern.

"The Hospital Situation."

A. "Scientific." By Robert A. Keilty, M.D., Washington, D. C.

B. "Statistical." By Philip B. Matz, M.D., Washington, D. C., read by Dr. A. H. Sanford, Rochester, Minnesota.

C. "Relation to the American College of Surgeons." By J. J. Moore, M.D., Chicago. Read by Dr. W. G. Gamble, Jr., Chicago.

Discussion by Dr. A. H. Sanford, Rochester; Dr. Frederic E. Sondern, New York City; Dr. Robert A. Keilty, Washington, D. C.; and Dr. F. W. Hartman, Detroit.

"Is the Cost of Laboratory Work Too High?" By Robert F. E. Stier, M.D., Spokane, Washington. Discussion by Dr. Mortimer Herzberg, Cincinnati, Ohio.

"The Clinical Pathologist in the Rural Hospital." By C. W. Maynard, M.D., Pueblo, Colorado. Discussion by Dr. W. T. Cummins, San Francisco; Dr. Zera E. Bolin, San Francisco; and Dr. Francis B. Johnson, Charleston, S. C.

Saturday Morning, July 6, 1929, 9 a.m.

The meeting was called to order by President Hartman and the scientific program continued:

"Gingivitis: V. The Character of the Exudate in Gingivitis." By Robert A. Keilty, M.D., Washington, D. C. Discussion by Dr. M. M. Patton, Seattle, Washington; Dr. F. H. Lamb, Davenport, Iowa; Dr. E. C. Rosenow, Rochester, Minnesota; Dr. F. W. Hartman, Detroit; and Dr. Robert A. Keilty.

"The Tuberculous Cavity." By Alfred Blumberg, M.D., Oteen, N. C. Read by Dr. E. R. Mugrage, Denver, Colorado. Discussion by Dr. F. W. Hartman, Detroit, Michigan, and Dr. Walter M. Simpson, Dayton, Ohio.

"Oxalic Acid as a Reagent for Isolating Tubercle Bacilli and a Study of the Growth of Acid-fast Nonpathogens on Different Mediums With Their Reactions to Chemical Reagents." By H. J. Corper, M.D. and Nao Uyei, Ph.D., Denver, Colorado. Discussion by Dr. Philip Hillkowitz, Denver; Dr. Frederic E. Sondern, New York; Dr. A. S. Giordano, South Bend, Indiana; Dr. E. C. Rosenow, Rochester, Minnesota; Dr. Robert A. Keilty, Washington, D. C.; and Dr. H. J. Corper.

"A Recently Isolated Bacillus of the Hemophilic Group." By Frank W. Hartman, M.D., and Edna Jackson, M.S., Detroit, Michigan. No discussion.

"Immunological Specificity of Green Producing Streptococci Having Elective Localizing Power as Isolated in Various Diseases." By E. C. Rosenow, M.D., Rochester, Minnesota. Discussion by Dr. Robert A. Keilty, Washington, D. C., and Dr. E. C. Rosenow.

Saturday Afternoon, July 6, 1929, 2 p.m.

The meeting was called to order by President Hartman and the scientific program continued.

"Milk Borne Rabies." By E. R. Mugrage, M.D., Denver, Colorado. No discussion.

"Observations on Intestinal Protozoiasis." By Rawson J. Pickard, M.D., San Diego, California. Discussion by Dr. Z. E. Bolin, San Francisco, and Dr. Rawson J. Pickard.

"Pathology of the Reticulo Endothelial System." By Zera E. Bolin, M.D., San Francisco, California. Discussion by Dr. E. R. Mugrage, Denver, and Dr. Z. E. Bolin.

"Reticulocytes, Their Identification and Significance." By C. L. Spohr, M.D., Columbus, Ohio. Read by title.

"Improved Colorimetric Procedures for the Quantitative Estimation of the Proteins of the Cerebrospinal Fluid." By Philip B. Matz, M.D., Washington, D. C., and Nathan Novick, Hines, Illinois. Read by title.

"Quantitative Microscopic Urinalysis." By William G. Exton, M.D., Newark, N. J. Discussion by Dr. Frederic E. Sondern, New York; Dr. E. R. Mugrage, Denver; Dr. Francis B. Johnson, Charleston, S. C.; and Dr. William G. Exton.

"New Quantitative Clinical Methods for the Junior Scopometer." 1. Protein in Urine, 2. Protein in Blood, 3. Protein in Spinal Fluid, 4. Globulin in Urine, 5. Sugar in Urine, 6. Sugar in Blood, 7. Urea in Urine, 8. Ammonia in Urine, 9. Creatinin in Urine, 10. Sulphur Partition in Urine. By William G. Exton, M.D., Anton R. Rose, Ph.D., and P. V. Wells, D.Sc., Newark, N. J. (Read by title.)

"Embryonal Carcinoma of the Testicle." By L. W. Larson, M.D., Bismarck, N. D.

"Malignant Tumors of the Testicle." By O. A. Brines, M.D., Detroit, Michigan. Discussion of both papers by Dr. Charles R. Drake, Minneapolis; Dr. Zera E. Bolin, San Francisco; Dr. Roy W. Hammack, Los Angeles; Dr. L. W. Larson and Dr. O. A. Brines.

Saturday Evening, July 6, 1929, 7 p.m. Annual Banquet

The Annual Banquet was held in the ballroom of the Portland Hotel. The speakers of the evening were as follows:

"Presidential Address." By Dr. Frank W. Hartman, Detroit, Michigan.

"Address." By Dr. R. G. Coffey, Portland, Oregon.

"Address." By Dr. Cyrus C. Sturgis, Ann Arbor, Michigan.

"Presentation of the Ward Burdick Research Award." The award was made by President Frank W. Hartman to Dr. Walter Malcolm Simpson, Dayton, Ohio, for his work in Tularemia.

Monday, July 8, 1929, 9 a.m. Business Session

The meeting was called to order by President Frank W. Hartman. The reading of the minutes of the previous meeting were dispensed with since they had previously been published.

The report of the Executive Committee was made by the Chairman, Dr. A. H. Sanford, Rochester, Minnesota. Dr. Sanford reported progress and that his Committee had selected Portland as the 1929 meeting place; they had inspected the audit of the books of the Treasurer and found it correct. Report accepted.

The report of the Editorial Committee was made by Dr. Robert A. Keilty, Washington, D. C., Associate Editor, by reading a communication from the Editor-in-Chief, Dr. T. B. Magath, Rochester, Minnesota, stating that our relations with the publishers of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE have been satisfactory, that they would like to receive editorials for the Journal from members of the Society, that the Round Table Discussions be abstracted for publication and that authors scrutinize their bibliographic references more carefully and abbreviate their manuscripts as much as possible. Report accepted.

The Report of the Publication Committee by the Chairman, Dr. John A. Kolmer, Philadelphia, was read by Dr. H. J. Corper, Denver, Colorado. This Committee reported progress made during the past year in the preparation of the book on Standard Methods in Clinical Pathology. Different sections will be submitted to different committees of the Society for revision so that the finished book will represent in truth and in fact methods approved by the Society. The authors hope the book will be authoritative and make the American Society of Clinical Pathologists' endorsement stand for the very highest in clinical pathology. Nothing further has been done regarding the publication of a journal by the Society but a letter from Williams and Wilkins Company shows their continued interest in this enterprise and offers their assistance in the project. Report accepted.

Dr. Philip Hillkowitz, Denver, Chairman of the Board of Registry, read the report of the Committee on the Registration of Technicians stating that steps had been taken to organize and formulate the plans for carrying the aims and purposes of the Registry into execution in accordance with instructions given at the last annual convention in Minneapolis. Application blanks had been drawn up; a booklet explaining the scope of the Registry was formulated and distributed widely; publicity was given the formation of the Registry by write-ups in the medical press of the country. There has been received up to this time over five hundred applications for registration which are being carefully examined and certificates will be issued to those successfully meeting the requirements of the Registry. The Committee is gratified at the favorable reception that has been accorded its efforts and the numerous letters of commendation and praise that have come both from applicants as well as clinical pathologists. Registration of approved training schools for technicians is also under consideration and a survey will shortly be made. The Employment Service in this connection has been functioning with success. A gold pin has been designed and which is available to those technicians meeting the requirements. Thanks were expressed to the members of the Society for their kind interest and cooperation, to the state representatives of the Registry and to the Board of Registry for the generous aid they have extended to the Chairman in the conduct of the Registry. This department has been a pronounced success both from the standpoint of the clinical pathologist as well as of the laboratory technician. Report accepted.

The report of the Committee on Public Relations by Dr. Edward F. Cooke, Chairman, Houston, Texas, was read by Dr. R. E. Myers, Oklahoma City, Oklahoma, and stated that many and serious evils are attacking the branch of medicine, clinical pathology, that it fails to attract young men; that many men in the field are leaving it for more lucrative fields. As a remedy for the Public Health Laboratory situation it was suggested that these laboratories should be restricted to strictly epidemiologic work, and that this work should come only through Public Health Officers and should not be available to any and every practitioner of medicine. Clinical pathologists have been approached by the American Medical Association to carry ads in the Medical Directory, yet no other branch of medicine would even be allowed to run such ads. The Committee also asks why the American Medical Association should approve laboratories when they take no steps like this in regard to any other specialty of medicine. An examination of the Medical Practice Acts of the forty-eight states reveals that in twenty-nine the statutes do not fully define the practice of medicine making it possible for an illegal practitioner to open a laboratory, charge for the laboratory examination, and give medical advice free. The use of the technician by physicians in their private laboratories is one of the most potent elements in the continuance of the evil, all this making clinical pathology an undesirable field for physicians. Resolutions drawn up by the Committee were read and referred to New Business. Report accepted.

The report of the Service Bureau Committee was made by Dr. H. J. Corper, Chairman, Denver, Colorado. Dr. Corper reported progress and expressed the conviction that in time this department of the American Society of Clinical Pathologists will be a very useful feature in placing our Fellows in the most desirable locations in the country and acting in a capacity of consultant and informant to the various institutions bringing their problems to us for solution. Report accepted.

The report of the Research Committee was made by Dr. A. S. Giordano, South Bend, Indiana, for the Chairman, Dr. Alvin G. Foord, Buffalo, N. Y., stating that this Committee endeavored to stimulate joint study of "Undulant Fever" and a symposium was planned for this meeting. In regard to the presentation of the Ward Burdick Award it was felt by the Committee that possibly it would be better to judge the papers as they are presented at the Convention but that the Executive Committee take the matter under consideration. The suggestion was made that the study of "Undulant Fever" be continued for another year in addition to some other subject. Report accepted.

The report of the Committee on Exhibits was made by Dr. C. H. Manlove, Chairman, Portland, Oregon. Dr. Manlove stated that their Committee had obtained eight exhibits in all and recommended that the next Chairman start his plans earlier. Report accepted.

The report of the Committee on Necrology was presented by Dr. J. H. Black, Chairman, Dallas, Texas, who reported the death of four Fellows of the American Society of Clinical Pathologists during the past year as follows: Dr. Joseph R. Losee, New York City, Dr. Carl O. E. Werner, San Francisco; Dr. John Hewat, New York City; and Dr. M. L. Holm, Lansing, Michigan. A motion was made and carried that the Society rise a moment in respect to the members that we have lost, and accordingly the members present arose. Report accepted.

The report of the Board of Censors was made by Dr. C. H. Manlove, Chairman, Portland, Oregon. The following were elected to Fellowship in the Society: Dr. Arthur L. Amolsch, Detroit; Dr. Walter G. Bain, Springfield, Illinois; Dr. O. A. Brines, Detroit; Dr. Henry T. Brooks, New York City; Dr. Theodore R. Helmbold, Pittsburgh; Dr. R. C. Henderson, Perry Point, Maryland; Dr. Frank P. Hunter, Lafayette, Indiana; Dr. Hugh Jeter, Oklahoma City; Dr. Frank L. Kelly, Philadelphia; Dr. Harry K. Langdon, Indianapolis, Ind. One reinstatement: Dr. W. W. Hall, Watertown, N. Y. One Associate Member, Dr. Willa M. F. Davis, Washington, D. C.

Under New Business was brought up the resolutions presented by the Committee on Public Relations. The first resolution stating that the Medical Practice Acts of twenty-nine states fail to properly define the Practice of Medicine as it exists today and in no state in the Union is there provision for any penalty for employing or covering illegal Practitioners of Medicine and resolves that the American Society of Clinical Pathologists in regular meeting assembled deplores this condition, calls the attention of the American Medical Association to it and urges relief. Resolution accepted. The resolution regarding Public Health Laboratories was defeated. A third resolution, that since there is an unfortunate tendency to regard clinical pathology as being independent and apart from the practice of medicine and to provide rules of ethics and conduct apart from those that are supposed to govern practitioners of medicine it is resolved by the American Society of Clinical Pathologists that clinical pathology is a legitimate special branch of medicine and those physicians who limit their work to clinical pathology are subject to the same code of ethics that governs all other physicians and that a copy of these resolutions be respectfully submitted to the American Medical Association. The resolution was passed with an amendment that the attention of the American Medical Association be called to the fact that they are soliciting advertising from clinical pathologists.

A letter from the Council on Medical Education and Hospitals of the American Medical Association was read by Secretary H. J. Corper inviting the American Society of Clinical Pathologists to be represented at their annual conference in February each year in the special conference on the subject of private clinical laboratories. A motion was made, seconded, and carried that the President of the Society be empowered to appoint three representatives of the Society to accept this invitation.

Secretary H. J. Corper read a letter from Mrs. Erna Burdick and Miss Georgiana Burdick expressing their appreciation of the institution of the Ward Burdick Research Award of the American Society of Clinical Pathologists.

The proposed amendments to the constitution and by-laws were read and adopted as follows:

ARTICLE III—MEMBERSHIP

Section 3

Associate members shall be graduates of recognized scientific institutions who have made such contributions to any of the sciences relating to clinical pathology and whose membership will so further the objects of the Society as to make them eligible for associate membership. Associate members shall pay the regular dues and have all the privileges of Fellows except those of voting and holding office.

Active members shall be designated as Fellows wherever that wording occurs, as follows: Article III, Section 1, line 2; Section 2, line 1. Article IV, Section 3, line 2; Section 4, line 2. Article VI, line 2. By-Laws, Article I, line 1. Article II, Section 1, line 1; Section 2, line 4. Article III, Section 1, line 1; Section 2, line 1. Article IV, Section 3, line 2. Article V, line 1. Article VII, Section 1, lines 5 and 6.

ARTICLE IV—OFFICERS

Section 1

The officers of this society shall consist of a President, President-Elect, Vice-President, Secretary-Treasurer, Executive Committee, Board of Censors, and Board of Registry of Technicians.

Section 5

The Board of Registry of Technicians shall be composed of six Fellows of the Society who shall each hold office for three years or until their successors are elected; two to be elected annually. The first Board shall consist of six Fellows, two of whom shall be elected for a term of one year, two for a term of two years and two for a term of three years. It shall elect its own Chairman from among the holdover members and Secretary-Treasurer.

Section 6

Vacancies in the interim on the Executive Committee, Board of Censors, or the Board of Registry of Technicians shall be filled by appointment by the President.

ARTICLE V—DUTIES OF OFFICERS

Section 6

The Board of Registry of Technicians shall conduct a Registry of Technicians, receive applications for such, pass on their qualifications and issue certificates to those meeting the requirements. They shall investigate schools for the training of technicians, registering those approved. They shall conduct a placement bureau for technicians.

A resolution was passed to extend a vote of thanks to the Local Committee on Arrangements for their good work in assuring the success of the Convention.

The election of officers for the ensuing year resulted as follows: President, Dr. J. H. Black, Dallas, Texas; President-Elect, Dr. Kenneth M. Lynch, Charleston, S. C.; Vice-President, Dr. H. H. Foskett, Portland, Oregon; Secretary-Treasurer, Dr. H. J. Corper, Denver, Colorado; Executive Committee, Dr. F. W. Hartman, Detroit, Michigan, three years, and Dr. Alfred S. Giordano, South Bend, Indiana, three years; Board of Censors, Dr. Frederick H. Lamb, Davenport, Iowa, three years, Dr. Warren T. Vaughan, Richmond, Va., three years; Board of Registry of Technicians, Dr. Philip Hillkowitz, Denver, Colorado, Dr. Kano Ikeda, St. Paul, Minnesota, three years; Dr. Alvin G. Foord, Buffalo, N. Y., Dr. C. Y. White, Philadelphia, Pa., two years; Dr. E. S. Maxwell, Lexington, Ky., Dr. William M. Thalhimer, Chicago, Illinois, one year.

The new President, Dr. J. H. Black, was inducted into office. Meeting adjourned.

The Journal of Laboratory and Clinical Medicine

VOL. XV ST. LOUIS, MO., JANUARY, 1930 No. 4

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - - - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - - - - - LOS ANGELES
J. J. R. MACLEOD, M.B. - - - - ABERDEEN, SCOTLAND
W. C. MACCARTY, M.D. - - - - - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - - - - - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - - - - - CLEVELAND
RUSSELL L. HADEN, M.D. - - - - - ROSEDALE, KAN.
JOHN A. KOLMER, M.D. - - - - - - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - - ATLANTIC CITY, N. J.
GEORGE HERRMANN, M.D. - - - - - - NEW ORLEANS
T. B. MAGATH, M.D. - - - - - - ROCHESTER, MINN.
DEAN LEWIS, M.D. - - - - - - - - BALTIMORE

Official Organ of the American Society of Clinical Pathologists and the American Association for the Study of Allergy

Contents of this Journal Copyright, 1930, by The C. V. Mosby Company—All Rights Reserved

Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

Multiple Malignant Neoplasms

MULTIPLE malignant tumors excite curiosity and stimulate interest in neoplasms. Basal cell epitheliomas are the most common of the multiple primary newgrowths. Owen, in 1921, found that next in order of frequency among multiple newgrowths, were combinations of basal cell and squamous cell carcinoma of the skin, and then, in succession, followed multiple squamous cell epitheliomas of the skin, multiple carcinomas of the breast, then carcinoma of the breast associated with some other type of carcinoma in some other organ.

The skin is the most common site for the development of multiple malignant neoplasms. Major, in 1918, observed that, disregarding the skin, multiple primary malignant tumors do not have a predilection for organs of the same system, except for paired organs, which is in contrast to their predilection for single organs. He observed that malignant newgrowths, again disregarding the skin, were more common in unrelated organs. When two related tumors are found in the same organ, one must answer the question: Is

this an example of metaplasia or are there two distinct primary tumors? Similar multiple malignant tumors in the same organ may be caused, for example, by carcinoma arising from several centers simultaneously or they may be merely a coincidence. Patients in whom multiple malignant tumors develop apparently have a neoplastic tendency which results in these numerous tumors.

Some of the peculiar features of multiple malignant neoplasms should be appreciated by clinicians, by pathologists and by surgeons. Application of such knowledge to the treatment of patients who present themselves with multiple malignant tumors will react to the advantage of patients and to the credit of those on whom responsibility for their treatment is imposed.

—*H. D. Caylor.*

Erratum

Referring to the article, "An Exploration Electrode to Determine the Hydrogen-ion Concentration of Fluids in Living Tissue," pp. 181-4 of the November, 1929, issue of this journal, certain essential data were not included and the author has submitted the following supplementary notes to be considered in connection with the original article.

The parts *A*, *B* and *C* in Fig. 1 of that article are ⅔ actual size and those in Fig. 2 are 1½ times actual size. The electrode wires must be 10-11 mm. long and not less than but not much more than 1 mm. thick. They must be of a platinum alloy rich in iridium and the wire should be polished until smooth. Platinum iridium electrodes are especially useful and reliable under trying conditions where electrodes made of pure gold or of platinum give variable and low readings.

This electrode is giving satisfactory results in our laboratory. It allows the determination of hydrogen-ion concentrations of viscous fluids, or solutions containing sediments and coarse suspended matter which would clog a foil electrode. Often even the P_H of a fluid which would poison a foil electrode can be obtained. The arrangement in Fig. 2 gives good results with thick, almost solid gels, by merely placing a drop of freshly boiled pure water at the point of contact. With the foil electrode the solution has to be saturated with hydrogen before equilibrium is reached and with increase in volume the time is proportionately longer. The correct E.M.F. with the point electrode is almost always obtained instantaneously, regardless of the amount of fluid used. With some solutions, especially those low in buffer, the E.M.F. has a tendency to drop and is highest only at the first moment of contact. On the other hand, with strongly buffered physiologic solutions, the P_H remains constant.

The values obtained with this electrode agree with the values obtained with the foil electrode for the whole P_H range.

The electrode is well adapted for P_H determinations on a large scale. Sixty tests an hour can readily be made with pure buffer solutions. Over five thousand E.M.F determinations have been made during the past year in this laboratory, where it has supplanted the foil electrode for almost all except electrometric titrations.

The American Society of Clinical Pathologists

Dr. J. H. Black, Dallas, Texas
President

Dr. Kenneth M. Lynch, Charleston, S. C.
President-Elect

Dr. H. H. Foskett, Portland, Ore.
Vice-President

Dr. H. J. Corper, Denver, Colorado
Secretary-Treasurer

Dr. T. B. Magath, Rochester, Minn., *Editor*
Associate Editors

Dr. Robert A. Keilty
Washington, D. C.

Dr. Charles R. Drake
Minneapolis, Minn.

Executive Committee:
Dr. F. W. Hartman, Detroit, Mich., Chairman.
Dr. A. H. Sanford, Rochester, Minn.
Dr. Frederic E. Sondern, New York
Dr. Wm. G. Exton, Newark, N. J.
Dr. John A. Kolmer, Philadelphia, Pa.
Dr. A. S. Giordano, South Bend, Ind.

Board of Censors:
Dr. F. H. Lamb, Davenport, Ia., Chairman.
Dr. C. H. Manlove, Portland, Ore.
Dr. Reuben Ottenberg, New York
Dr. Ernest Scott, Columbus, Ohio
Dr. B. W. Rhamy, Fort Wayne, Ind.
Dr. Warren T. Vaughan, Richmond, Va.

Past Presidents

Dr. Philip Hillkowitz, Denver, Colo. 1922-3
Dr. Wm. Carpenter MacCarty, Rochester, Minn. 1923-4
Dr. John A. Kolmer, Philadelphia, Pa. 1924-5
Dr. Frederic E. Sondern, New York, N. Y. 1925-6
Dr. Wm. G. Exton, Newark, N. J. 1926-7
Dr. A. H. Sanford, Rochester, Minn. 1927-8
Dr. F. W. Hartman, Detroit, Mich. 1928-9

News and Notes

To the Members of the American Society of Clinical Pathologists:

By unanimous agreement of the Research Committee, President Black, and members of the Executive Committee, it has been decided to use the subject, "Agranulocytosis," as the topic for the symposium at the Detroit meeting, discussing it chiefly as the condition first described by Schultz as "Agranulocytic Angina," but also including those cases with lesions elsewhere than in the mouth, and also cases with blood pictures showing agranulocytosis along with other features in the blood picture or etiology to exclude the diagnosis of agranulocytic angina of Schultz. The members of the Society are earnestly requested to summarize their findings in cases observed by them in recent years so that the information from all may be presented at the next meeting. To facilitate the reporting of cases, questionnaires will be sent from the Secretary's office some time in the spring of 1930, but meanwhile it is hoped that cases studied in the past and those in the next few months will be completely worked up preparatory to summarizing in the questionnaire.

The united efforts of the membership are also asked for in the study of the question as to the avian tubercle bacillus being the cause of Hodgkin's disease as suggested by Dr. L'Esperance from her chicken inoculation experiments which she reported before the last two meetings of the Society of American Pathologists and Bacteriologists. By pooling the results of well controlled experiments conducted by our members throughout the country, some concrete idea as to the rôle played by the avian strain can be formulated.

Further endeavor to uncover undulant fever cases and to carry on the work begun last year appears also to be quite in order and again we hope to accumulate more data from our members in all the types of laboratories served by them. Please keep this subject in mind as one of the problems to be studied as a joint effort of the Society.

Trusting that you can contribute personally to all of the three problems, we remain

Fraternally yours,

Research Committee,
Alvin G. Foord, Chairman,
Buffalo General Hospital, Buffalo, New York.